Blood rheology and thrombotic mediators in peripheral arterial disease and revascularisation surgery.

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TABLE OF CONTENTS **Page**

LIST OF TABLES

LIST OF ILLUSTRATIONS

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DECLARATION

"I declare that the contents of this thesis, submitted to the University of Edinburgh for the degree of Doctor of Medicine, were composed entirely by myself. This thesis is based entirely on my own observations and, except as indicated in the text, the experiments were carried out, the data were collected, and the results were analysed and interpreted by myself."

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This thesis is dedicated to my wife and family for all their patience, understanding, and support.

PREFACE

The surgical treatment of peripheral arterial disease has progressed significantly over the past 40 years. Newer non-invasive methods of treatment have augmented traditional open surgical procedures, and in recent years blood rheology, thrombotic mediators, and white blood cell activation have all been associated with peripheral arterial disease.

This thesis was undertaken in an attempt to define more clearly the role of some of these variables in peripheral arterial occlusive disease. In particular I aimed to determine if alterations in blood rheology and potential thrombotic mediators were related to the severity of arterial disease; and whether or not they were affected by therapeutic intervention in the form of infra-inguinal bypass grafting. The ability to readily determine the patency of these grafts by a variety of non-invasive techniques has enabled accurate determination of the outcome of infra-inguinal grafting. Therefore baseline levels and serial changes in blood rheology and thrombotic mediators following revascularisation were related to the outcome following surgery in a large number of patients.

In addition I was interested in the effects of percutaneous angioplasty on these variables, as this procedure is now the main treatment in patients with an appropriate distribution of disease, and the response of the blood to angioplasty, which causes endothelial injury, may offer clues to the aetiology of arterial lesions in peripheral arterial disease.

It is to be hoped that a greater understanding of the biochemistry of peripheral arterial disease, both prior to and following revascularisation procedures, may lead to new therapeutic approaches offering a means of improving the results of such procedures.

ABSTRACT

Rheological parameters and levels of plasma fibrinogen, cross-linked fibrin degradation products (FDP's), von Willebrand Factor antigen (vWF), factor VII, and Plasminogen Activator Inhibitor (P.A.I.), were found to be altered in 219 patients with occlusive arterial disease undergoing revascularisation procedures, when compared with an age-matched random population sample. Elevated levels of some of these potential thrombotic mediators were associated with the angiographic severity of arterial disease on univariate analysis, while multivariate analysis indicated that log(FDP) was independently related to disease severity, while other thrombotic mediators showed a non-significant trend towards independent association.

Surgical relief of critical limb ischaemia in 82 of these patients returned the alterations in blood rheology to control levels, but plasma fibrinogen, vWF, P.A.I., and FDP levels remained significantly higher than in the population controls.

Percutaneous angioplasty in 40 cases was associated with transient alterations in vWF and Tissue Plasminogen Activator (t.P.A.) levels in arterial blood that may be a consequence of endothelial injury, and with a significant elevation in cross-linked FDP levels 3 months after successful angioplasty. This alteration in fibrin turnover may be related to restenosis following angioplasty.

Elevated pre-operative plasma fibrinogen, vWF, and FDP's, were associated with graft occlusion and patient death on univariate analysis of 186 consecutive infra-inguinal bypass grafts. Multivariate analysis indicated that pre-operative plasma von Willebrand Factor levels ($p < 0.001$), systolic ankle pressure ($p < 0.01$), female sex ($p = 0.03$), and limb sepsis ($p = 0.01$), were all independently predictive of graft occlusion and patient death within 1 year following surgery.

The studies undertaken in this thesis indicate that altered levels of potential thrombotic mediators found in peripheral arterial disease are not reversed by limb revascularisation, and that more marked alterations are associated with a poor outcome following surgery. The results suggest that alterations in levels of potential thrombotic mediators are not a consequence of tissue ischaemia, and may be directly involved in the pathogenesis of arterial occlusion. Pre-operative determination of these mediators may assist in patient selection for infra-inguinal revascularisation surgery, while adjunctive therapy aimed at correcting these abnormalities may improve the results of such surgery.

CHAPTER 1

General Introduction

INTRODUCTION

Peripheral vascular disease encompasses the clinical conditions that arise as a consequence of the thickening and hardening, or "arteriosclerosis", of major peripheral arteries. There are a number of pathological processes that lead to arteriosclerosis, but the commonest is atheroma, which can give rise either to narrowing and eventual occlusion of vessels, or to dilatation and aneurysm formation (Walter & Israel, 1987B). It is this narrowing of major peripheral vessels by atheroma that leads to the development of peripheral arterial occlusive disease (PAOD).

A significant number of the population with objectively defined peripheral arterial occlusive disease are asymptomatic, or fail to consult a medical practitioner with their symptoms (Hughson et al, 1978, Fowkes et al, 1991). Those patients who present with symptomatic occlusive arterial disease, usually present with the symptoms of chronic ischaemia, ranging from intermittent claudication through rest pain to tissue necrosis and gangrene.

Most patients with PAOD who consult a medical practitioner, present with a history of leg pain on exertion that is relieved immediately on stopping walking (**intermittent claudication**) (Hertzer, 1991). This pain characteristically recurs at a similar distance each time walking is resumed (Clain, 1986). The distance walked to the onset of pain (the claudication distance) can be used as a rough guide to the severity of the arterial disease.

Intermittent claudication commonly occurs in the calf, but can also occur in the thigh or buttock, depending on the distribution of the arterial occlusive lesions, which have been shown to be in the superficial femoral or popliteal segments in up to 70% of patients (Bloor, 1961). The pain in claudicants reflects the inability of a diseased peripheral arterial tree to meet the increased blood flow requirements of exercising muscles, resulting in the accumulation of metabolites, possibly kinins, that stimulate pain receptors in the muscles (Cotton, 1983).

More advanced occlusive arterial disease presents with more advanced features of tissue ischaemia, which ultimately lead to tissue necrosis and **gangrene** (Figure 1.1). These patients often give a history of prior intermittent claudication before presenting with calf and foot pain at rest, typically more severe at night as a consequence of the decreased cardiac output during sleep, and the increased metabolic demands of tissues that have been warmed under the blankets (Clain, 1986). This **rest pain** may be temporarily relieved by adopting the dependent posture, but these symptoms of chronic pain in patients with occlusive arterial

Figure 1.1: Gangrenous foot resulting from peripheral arterial occlusive disease.

disease herald the onset of **critical limb ischaemia** (European Working Group on Critical Limb Ischaemia, 1990), which if untreated will progress to tissue necrosis and gangrene.

A small number of patients present with an acutely ischaemic limb: a painful white leg associated with absence of peripheral pulses and diminished skin sensation in the limb. These patients have sustained an acute occlusion of a major limb vessel in the absence of the adequate collateral flow, which commonly develops in chronic occlusions (Krupski & Effeney, 1988). In these situations, prompt revascularisation is required to salvage the limb.

Patients with acute arterial occlusion have either occluded a relatively healthy vessel with embolic material from a distant source such as atrial thrombi in patients in atrial fibrillation (Cotton, 1983), sustained a haemorrhagic dissection beneath a pre-existing plaque (Krupski & Effeney, 1988), or have developed further thrombosis on pre-existing atheroma (Walter & Israel, 1987B).

The first group of patients, who account for up to 75% of patients presenting with acute lower limb ischaemia (Clason et al, 1989), can be revascularised by early Fogarty embolectomy, with few long-term sequelae. The remaining patients however, have significant underlying occlusive arterial disease, and the development of acute critical limb ischaemia makes early revascularisation surgery mandatory if the limb is to be saved.

EPIDEMIOLOGY OF PERIPHERAL ARTERIAL DISEASE

Incidence and prevalence

The true prevalence of peripheral arterial disease in the community is poorly documented, although recent population studies suggest a prevalence rate for intermittent claudication of between 2% and 14% depending on the population studied and the age of the study group (Fowkes, 1988, Fowkes, 1990, Gillum, 1990). The Edinburgh Artery Study (Fowkes et al, 1991) found a prevalence rate for intermittent claudication of 4.5% in a cross section of the Edinburgh population aged 55 to 74 years. In addition the study found that 8% of the population had evidence of major asymptomatic disease, as determined by resting ankle brachial pressure indices (ABPI) of less than 0.9, together with a negative response to the W.H.O. questionnaire on intermittent claudication. This combined figure of 12.5% of the population with a haemodynamically significant impairment of peripheral

circulation represents the only currently available estimation of the disease prevalence in a Scottish population.

These figures are comparable to the prevalence rate for peripheral arterial disease of 11.7% reported in a geographically defined white population in Southern California (Criqui et al, 1985). The prevalence of symptomatic disease as manifest by intermittent claudication, was however lower at 2.2% for men and 1.7% for women, and a similar incidence of claudication was reported in an English study (Hughson et al, 1978) in which the diagnosis was based on a history of leg pain on exertion, and a resting ABPI of less than 0.75. However a resting ABPI of less than 0.9 has been shown to be 95% sensitive in detecting disease (Laing & Greenhalgh, 1983), suggesting that this study underestimated the incidence of intermittent claudication in the population examined.

All the population studies that have examined the incidence and prevalence of peripheral arterial disease have noted that both symptomatic and asymptomatic disease increase with age, and that, although the prevalence of disease in younger people is higher in men, the prevalence rate is similar for both sexes in older populations (Rose, 1991).

Aetiological factors

Epidemiological and population studies have identified a number of factors that appear to be related to the development of peripheral vascular disease. A high level of **cigarette smoking** is the most consistent finding amongst patients with peripheral arterial disease, and the relative risk of disease in smokers compared with non-smokers ranges from 1.4 to 7.5 (Fowkes, 1988). Persistent smoking has also been associated with graft failure following reconstructive surgery (Wiseman et al, 1989).

Diabetes mellitus is associated with an increased risk of developing arterial disease (Walter & Israel. 1987B), but additionally, some studies have suggested that patients with **glucose intolerance** are at increased risk of developing peripheral arterial disease (Fowkes, 1988), however there are conflicting results from other studies (Hughson et al, 1978).

Elevated **serum cholesterol** and **triglyceride** levels in patients with peripheral arterial disease, have been demonstrated in some studies, although there are conflicting results (Greenhalgh et al, 1971, Kakkar & Stringer, 1990, Leng and Fowkes, 1991B), despite evidence that lowering serum lipid levels leads to regression in peripheral atherosclerosis (Barndt et al, 1977, Duffield et al, 1983). There is stronger evidence that low **HDL cholesterol** levels are an important risk

factor (Meerloo & Billimoria, 1979, Leng & Fowkes, 1991B), and also evidence that **lipid peroxides** are elevated in patients with arterial occlusive disease (Stringer et al, 1989). Some of the changes in the lipid profiles of patients with peripheral vascular disease may however be related to cigarette smoking (Craig et al, 1989).

There are some studies that suggest a role for **hypertension** in the aetiology of peripheral arterial disease (Hughson et al, 1978), however this has not been a uniform finding (Fowkes, 1988). The potential aetiological roles of **fibrinogen** and other **haemostatic and rheological factors** are covered in more detail elsewhere in this chapter.

NATURAL HISTORY OF PERIPHERAL VASCULAR DISEASE

The natural history of asymptomatic peripheral arterial disease is poorly documented, although there is some evidence from small studies to suggest that between 30 and 47% of asymptomatic patients will develop intermittent claudication within 4 years of the disease being detected (Strandness 1966, Laing & Greenhalgh, 1983). The natural history of intermittent claudication, and the fate of claudicants is however, more fully documented.

Mortality

The finding common to all studies of the natural history of intermittent claudication, is the greatly increased mortality in claudicants (Table 1.1), as a consequence of myocardial ischaemia and cerebrovascular events, and claudicants have a mortality rate twice that of the normal population (Kannel et al, 1970, O'Riordan & O'Donnell, 1991). The 5 year survival for the claudicant aged 65-74 appears to be around 60%, dropping to 20% at 10 years (Bloor, 1961), and the overall mortality rate for any group of claudicants is 30-35% at 5 years (Rosenbloom et al, 1988, O'Riordan & O'Donnell, 1991), and 50% at ten years (Dormandy, 1991). The mortality rate for patients presenting with more advanced disease in the form of critical limb ischaemia is higher than that for claudicants, with a 5 year mortality rate of 50% (Norgren, 1990).

Disease progression

A patient with intermittent claudication surviving 5 years, has a 25% chance of developing a further "clinically detectable" arterial occlusion (Bloor, 1961) and

STUDY	No. of patients	Mortality rate
Bloor (1961)	1,476	46% at 10 yrs.
Singer & Rob (1960)	322	23% at 5yrs.
Kannel et al (1970)	125	x2 over 14 yrs
Rosenbloom et al (1988)	378	50% at 8 yrs.
Dormandy (1991)	1,969	4.3% per annum
O'Riordan (1991)	112	33% at 5yrs.

Table 1.1: Crude mortality rates in patients with intermittent claudication.

a 7% chance of requiring above-knee amputation. In the same study over 50% of surviving claudicants noticed an improvement in their symptoms, while over a 3 year period, 56% of claudicants in one study improved or remained static (Singer & Rob, 1960).

Other reports of the fate of patients with symptomatic occlusive arterial disease show a similar pattern of events (McDaniel & Cronenwett, 1989), while a recent prospective study found a major amputation rate of 1.6%, and an intervention rate of 5.6%, in 1969 claudicants over 1 year (Dormandy & Murray, 1991). Over a longer time period approximately 1 in 5 claudicants will suffer from worsening claudication, while 1 in 8 may develop critical ischaemia (O'Riordan & O'Donnell, 1991). A higher rate (41% over 8 years) for the development of critical limb ischaemia has however been reported in a series with an unusually high (34%) percentage of diabetics (Rosenbloom et al, 1988). Overall however the figures from a number of series suggest that in a 5 year period only 29% of claudicants will require surgery, including 4% who will require an amputation.

Site of disease

The natural history of peripheral arterial disease at different anatomical sites is poorly documented, although in a study of superficial femoral disease (Walsh et al, 1991), 28% of asymptomatic and mildly symptomatic superficial femoral artery stenoses progressed over a 3 year period, with 17% occluding. This was a small study in a rather atypical population however, with no objective assessment of the peripheral circulation following disease progression, and until further larger studies are carried out, accurate data on the rate of progression of arterial disease at different sites remains unavailable. Current treatment plans for patients with intermittent claudication have therefore to be based on the data already described, and on the preference of the individual clinician.

Summary

Based on the data currently available, in a typical group of 100 symptomatic claudicants aged 55-65 years, 30 of these patients would be expected to die in the 5 years following presentation (Figure 1.2), while of the remaining 70 patients, around 50 will have improved or stable claudication distances. This leaves 35 patients whose symptoms will deteriorate, although only 10 patients are liable to develop critical limb ischaemia, with 2 or 3 coming to amputation in the 5 years following presentation, and the remainder undergoing surgery for limb salvage.

There remain 25 of the original 100 patients whose symptoms will have

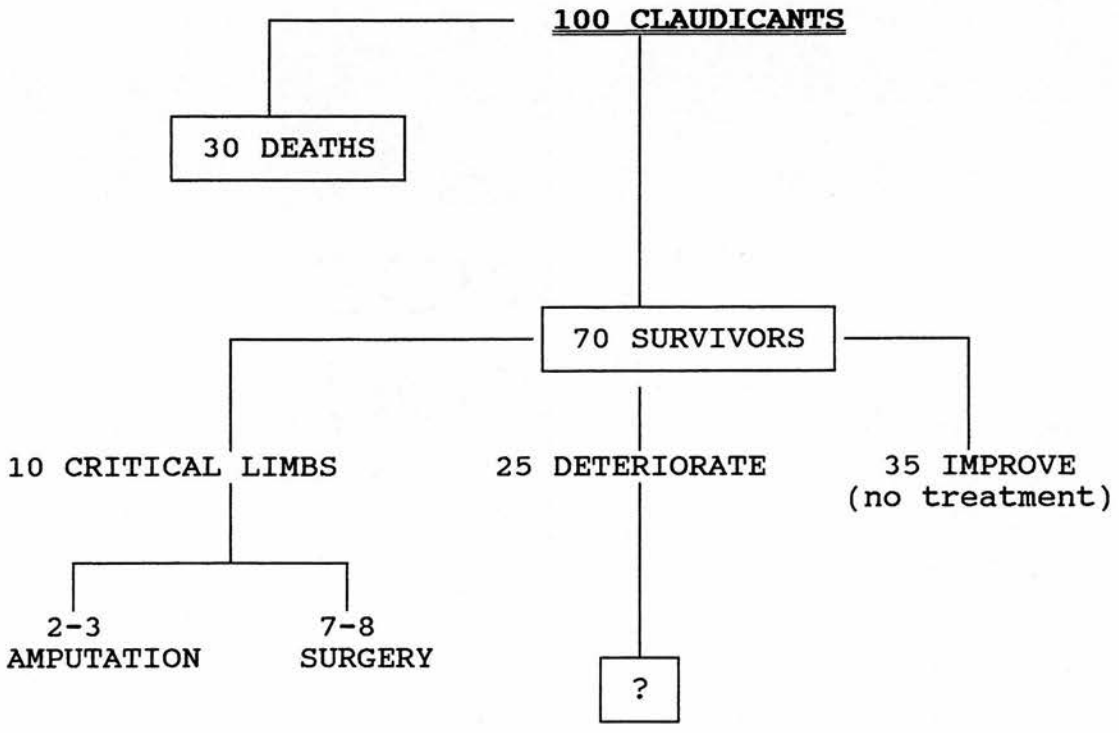


Figure 1.2: The typical 5 year outcome in 100 patients presenting with intermittent claudication.

deteriorated to a point short of critical ischaemia. Surgery in this group will be determined by the distribution of their disease, the severity of their symptoms, and their (and their surgeon's) attitude to operation. However at the current time, there are no means of accurately predicting which group any individual patient will fall into when they first present with symptomatic peripheral arterial disease.

Alternatively, if the surgeon operates on all 100 claudicants presenting to his clinic, he will operate on 35 patients whose symptoms would have improved without operation, and on 30 patients in whom the period of benefit, if any, would have been short lived, due to co-existing, and ultimately fatal, cardiac or cerebrovascular disease. This, together with the not insignificant failure rate of reconstructive surgery for infra-inguinal disease (Bell 1990), makes patient selection important, with the emphasis on identifying those patients who will achieve most benefit from surgery, with the least risk of mortality, and the lowest graft failure rate.

INVESTIGATION OF PERIPHERAL VASCULAR DISEASE

Most patients with symptomatic peripheral arterial occlusive disease can be diagnosed on the basis of a history of intermittent claudication, the characteristic findings of the absence of one or more peripheral pulses, and the presence of thrills or bruits associated with turbulent flow in certain cases (Clain, 1986). These findings, together with the more obvious features of limb ischaemia found in more advanced cases (Figure 1.1), enable a confident diagnosis of arterial insufficiency to be made in most cases, however there are a number of additional investigative techniques that are usually undertaken:

Ankle-Brachial Pressure Indices

The use of ultrasonic flow detectors to determine the systolic pressure in ankle vessels was described by Yao in 1968 (Yao et al, 1968), and subsequent to this it was reported that a pressure index obtained from the ratio of ankle systolic pressure to brachial systolic pressure, correlated well with arterial stenosis and occlusion, the ratio in normal patients being greater than 1.0, and in patients with peripheral arterial occlusive disease, less than 1.0 (Yao et al, 1969).

This ratio, which has come to be known as the Ankle-Brachial Pressure Index (ABPI), remains the simplest and most effective means of determining the presence of occlusive arterial disease, with a ratio of 0.9 or less diagnostic of

arterial insufficiency (Laing & Greenhalgh, 1983). The ABPI has a low coefficient of variation (Horrocks & Scott, 1991), and can be used to monitor the progress of the disease. The ABPI value may however be misleading in patients with arterial wall calcification, as found in diabetes mellitus and end-stage renal disease (Barnes, 1991). The addition of an exercise test, with measurement of the ABPI after a standard exercise regime further improves the diagnostic sensitivity of the test (Nicolaides, 1991), with a drop in the ABPI following exercise confirming the presence of occlusive disease. In patients unable to carry out an exercise test, this effect can be reproduced using an occlusive cuff hyperaemia test (McShane et al, 1988), which has been found to correlate well with the response to a standard exercise test (Wyatt et al, 1990).

Doppler blood velocity tracings

A number of authors have investigated methods of analysing the Doppler velocity waveform. Simple analysis of the waveform (Yao, 1970) can help in the localisation of significant stenoses, and more detailed mathematical analysis of the same waveform may detect aorto-iliac disease (Nicolaides et al, 1976). Pulsatility index (PI) calculations (Gosling et al, 1971), Laplace transform of the peak velocity waveforms (Baird et al, 1980), and principal component analysis of spectrum analysed continuous wave Doppler (MacPherson et al, 1984) have also been suggested as means of assessing the extent of arterial occlusion, but all these techniques require a skilled technician, and often the use of sophisticated computers. As a result most of these techniques have remained as research tools, and play little part in the routine evaluation of the vascular patient.

Duplex scanning

In the early 1970s ultrasonic imaging techniques were developed and subsequently combined with Doppler ultrasonic devices to create the Duplex scanner, which provided both anatomical and functional information about the peripheral circulation. Real-time B-mode imaging of vessels enables identification of anatomical lesions, and also ensures that the Doppler signal that is being analysed is from the desired vessel, as the point of sampling for the signal is selected by the operator. More recent versions of these machines are able to display some of the Doppler data as a colour display of flow superimposed on the real-time image (Allan, 1991).

Colour flow duplex devices enable visualisation of some arterial lesions together with assessment of their functional significance, in terms of velocity,

diameter, and flow (Renton & Nicolaides, 1991). There is now some evidence that duplex is as effective as arteriography in the detection of haemodynamically significant lesions in both aorto-iliac and femoro-popliteal segments (Jager et al, 1985, Legemate et al, 1991). However the role of colour flow duplex in the investigation of occlusive arterial disease is not yet clearly established, although it would appear to have a role in graft surveillance (Fig. 1.3) (Grigg et al, 1988B, Harris, 1992). The main disadvantages of the technique are that visualisation and acquisition of Doppler information from some segments is difficult to obtain (in particular from iliac and calf vessels), and that information obtained often represents a compromise between image quality and Doppler information (Allan, 1991).

Arteriography

Since 1924, when Brooks (1924) described the radiological imaging of peripheral arteries using intra-arterial injection of sodium iodide, the technique of arteriography has been the accepted "gold standard" for investigation of peripheral vascular disease. However it was not until 1953 that relatively safe percutaneous catheterisation of the arterial tree became available (Seldinger, 1953), and the technique of percutaneous catheterisation introduced by Seldinger remains the method of choice for obtaining angiography.

Intra-arterial injection of radiopaque contrast material enables the entire vascular anatomy of the lower limbs to be visualised in a single examination, while serial arteriography enables the clinician to monitor the progress of specific lesions. Newer computerised imaging techniques such as digital subtraction angiography (DSA) have improved the quality of images available, and reduced the need for large doses of intra-arterial contrast media (Sutton, 1990), however arteriography remains an invasive technique that is not devoid of complications (Sutton, 1990).

The major disadvantage of arteriography in clinical practice is that the routinely performed uniplanar views may fail to demonstrate some significant lesions, in particular in the aorto-iliac segments, where lesions that are only affecting the lumen in an antero-posterior plane will not be visualised. In addition, arteriography gives no functional information about the lesions demonstrated.

Summary

History and clinical examination alone are often sufficient to determine the presence of significant arterial occlusive disease, but further information is usually sought before a plan of management is devised for any given patient. This usually

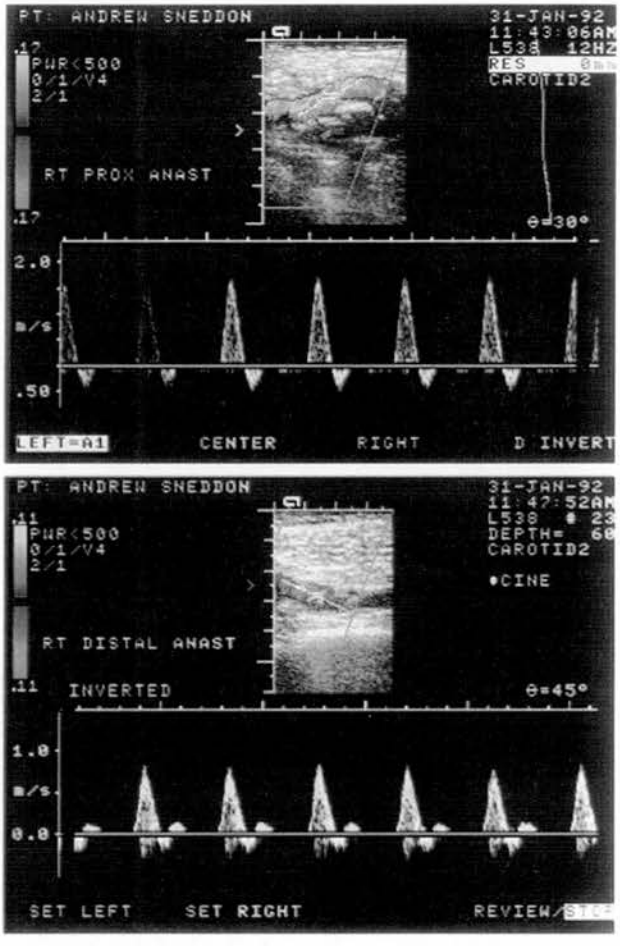


Figure 1.3: Duplex scans of left femoropopliteal graft showing groin vessels and popliteal anastomosis.

consists of semi-quantitative assessment of disease severity (ABPI), and radiological imaging of the arterial tree by conventional arteriography or DSA. Duplex ultrasonography now offers a non-invasive means of obtaining similar information in some situations, but has yet to gain widespread acceptance, and has limitations that will ensure that arteriography remains an important diagnostic tool in the foreseeable future.

TREATMENT OF PERIPHERAL VASCULAR DISEASE

Until the middle of the 20th century treatment of symptomatic arterial occlusive disease was confined to amputation of the affected limb when gangrene had developed. Since then a number of different methods of treating the patient with symptomatic arterial occlusion have been developed, although amputation may well still be required in some cases.

Medical therapies

Medical therapies for peripheral arterial occlusive disease are aimed at reducing risk factors, improving collateral circulation, and avoiding foot trauma (Krupski & Effeney, 1988). In many cases these measures are enough to enable the patient with PAOD to lead a symptom-free existence, with as many as 50% of claudicants remaining stable or improving in the 5 years following presentation (Singer & Robb, 1960, Bloor, 1961, McDaniel & Cronenwett, 1989), and most claudicants can be treated initially with this simple advice on lifestyle modification (Housley, 1988).

Risk factors such as smoking, hypertension, hyperlipidaemia and polycythaemia should all be treated, and good diabetic control encouraged. Smoking however is the only risk factor that has been consistently related to intermittent claudication in most studies (Davey-Smith et al, 1991), and the benefits of altering other risk factors associated with occlusive arterial disease remain to be proven.

Despite the lack of controlled studies, walking exercise is thought to stimulate the development of collateral flow, with a consequent increase in the claudication distance (Krupski & Effeney, 1988). There is some evidence to suggest that there is a link between low physical activity in middle-age, and the subsequent prevalence of PAOD (Housley, 1991), while a significant improvement

in maximal and pain-free walking distances in stable claudicants following 2 months of treadmill exercising has been reported (Ernst & Matrai, 1987).

These simple measures are often enough to produce symptomatic relief in many patients with occlusive arterial disease, but in patients whose claudication is significantly interfering with their lifestyle, or in whom there is evidence of limb threatening ischaemia, additional measures are required (Housley, 1988).

Pharmacological therapies

A number of different drug therapies have been evaluated in peripheral vascular disease, either in an attempt to alter the course of the disease, or to reduce the associated cardiac and cerebrovascular mortality and morbidity in patients with peripheral vascular disease. However the evaluation of the benefits of drug therapy in intermittent claudication is complicated by the 50% rate of spontaneous improvement shown in claudicants over a period of time (McDaniel & Cronenwett, 1989), and by effects of any associated reduction in smoking and increase in exercise.

The two drugs most commonly prescribed in arterial occlusive disease, **naftidofuryl oxalate** (Praxilene, Lipha) and **pentoxifylline** (Trental, Hoescht), have both shown a small improvement in the walking distance of patients with claudication in some studies (Trübestein et al, 1984, Rössner & Müller, 1987), but the small numbers studied create difficulties in interpreting the results (Bollinger & Frei, 1977), and meta-analysis has been required to produce statistically significant results (Lowe, 1990B). The mechanisms by which both these drugs produce their therapeutic effects are somewhat unclear, although some possible mechanisms of action have been demonstrated for pentoxifylline (Currie et al, 1991).

Vasodilator drugs which either act directly on vascular smooth muscle or indirectly by actions on the sympathetic nervous system, may theoretically be of benefit in arterial occlusive disease: drugs acting on the sympathetic nervous system may increase skin blood flow, but will not increase blood supply to ischaemic muscle (Coffman, 1979), while drugs that directly relax vascular smooth muscle have not been shown to improve the blood supply to ischaemic tissue despite an increase in calf blood flow (Coffman, 1979, Morgan et al, 1987).

Antiplatelet therapy has been shown to be protective in patients with cerebrovascular and coronary artery disease, (Antiplatelet trialists collaboration, 1988), and also to reduce the rate of occlusion of coronary artery grafts (Petch, 1991). Although the role of platelets in peripheral arterial disease is still unclear, there have been studies of antiplatelet therapy in PAOD that have suggested that a

similar beneficial effect may exist (Bounameaux et al, 1986). In a prospective double-blind arteriographically controlled trial of both aspirin and dipyridamole (Hess et al, 1985), a beneficial effect in terms of reduced disease progression was shown for antiplatelet therapy in smokers and hypertensives, while the anti-platelet agent ticlopidine (an inhibitor of ADP-induced platelet aggregation), has been shown to improve walking distance, walking speed, and ABPI, in patients with symptomatic occlusive arterial disease, as well as reducing cardiac and cerebrovascular events in claudicants (Janzon et al, 1990, McTavish et al, 1990). Aspirin and dipyridamole in combination have also been shown to reduce myocardial infarction and stroke rate following reconstructive vascular surgery (McCollum et al, 1991A).

Arterial thrombosis is often the final step in PAOD, and it may be that **anticoagulant therapy** can influence the course of the disease, although the evidence is inconclusive (Bounameaux et al, 1986). A small study in patients with advanced arterial disease suggested that the reduction in plasma fibrinogen, plasma viscosity, and blood viscosity, following treatment with subcutaneous ancrod, was associated with increased nutritional skin blood flow, however large clinical trials have not been performed (Lowe et al, 1979). The use of minidose warfarin in claudicants merits further evaluation (Reid, 1991). The use of full dose anticoagulants in patients with occlusive arterial disease is restricted to patients in whom there is a clear clinical indication for anticoagulant therapy.

Administration of **prostacyclin (PGI_2)** by either the intra-arterial or intravenous route, which results in vasodilatation, dispersion of circulating platelet aggregates, and inhibition of platelet aggregation, has been shown to reduce levels of ischaemic rest pain in advanced occlusive arterial disease and improve the healing of ischaemic ulcers (Szczeklik et al, 1979, Belch et al, 1983), while intravenous infusion of the prostacyclin analog **Iloprost** (Schering A.G.) has been shown to have similar beneficial effects in patients with advanced non-reconstructible arterial disease, with a significant improvement in clinical status and reduction in amputation rate (UK Severe Limb Ischaemia Study Group, 1991). Intravenous Iloprost is also of value in patients with thrombangitis obliterans (Fiessinger & Schafer, 1990), but the exact role of prostaglandins in the treatment of peripheral arterial occlusive disease has yet to be determined.

Drug treatment of occlusive arterial disease is therefore only an option for a limited number of patients with limb threat, who may gain some benefit from intravenous prostaglandin therapy. There are no drug therapies that have convincingly shown a clear benefit in intermittent claudication, although

antiplatelet agents may be of value in reducing the cardiovascular morbidity and mortality associated with PAOD (McTavish et al, 1989, McCollum et al, 1991A).

Percutaneous arterial recanalisation

Since the advent of the Seldinger technique to place intravascular devices (Seldinger, 1953), the potential has existed to effect percutaneous treatment of arterial stenoses and occlusions. Early methods of dilating arterial stenoses involved the passage of increasing sizes of dilating catheters over an appropriately placed guidewire, thus enlarging the arterial lumen (Dotter & Judkins, 1964). However it was not until the development of a catheter with an inflatable balloon, that successful percutaneous dilatation could be achieved through a small arterial puncture with good results and a low complication rate (Gruntzig & Kumpe, 1979). Since then **balloon angioplasty** has become the mainstay of percutaneous treatment for peripheral arterial occlusive disease, and it is now being used to treat patients in whom the risks of surgery would not normally be justified (Campbell, 1986, Michaels, 1990, Whyman et al, 1991).

The results of balloon angioplasty are variable (Michaels, 1990) and are greatly influenced by the nature of the lesion being treated. Lesions involving the iliac arteries have an initial technical success rate of over 90%, angioplasty of femoral lesions is successful in between 70 and 85% of cases (Gruntzig & Kumpe, 1979, Freiman et al, 1979, Spence et al, 1981, Waltman et al, 1982, Johnston et al, 1987) and the 1-year patency rate following angioplasty of the popliteal artery and tibial stem is somewhat lower at 57% (Tamura et al, 1982). Long-term results are less encouraging, with 5-year patency rates of around 64% for iliac lesions and 40% for femoro-popliteal lesions (Johnston et al, 1987), in contrast to cumulative patency rates for aortic bifurcation grafts of up to 99% at 5 years (Reid & Pollock, 1991), and a 3 year patency of between 55 and 80% for femoro-popliteal grafts (Cheshire & Wolfe, 1992).

Intermediate patency rates at 2 and 3 years of 80-90% for iliac lesions, and 60-70% for femoro-popliteal lesions are quoted in a number of series (Gruntzig & Kumpe, 1979, Freiman et al, 1981, Waltman et al, 1982), but comparison between studies is complicated by the wide variations in the patient population, and definition of success (Cambria et al, 1987, Rutherford & Becker, 1991), although it is accepted that patency declines for a number of years following successful angioplasty (Johnston et al, 1987). Failure rates for angioplasty of the femoropopliteal segment however appear to be highest within the first year (Martin et al, 1981, Mosley et al, 1985).

A number of factors that influence the outcome of angioplasty have been identified: any stenoses or occlusions longer than 10cm have a poorer outcome, as do patients in whom the procedure is carried out for limb-threat, or with a poor run-off (Spence et al, 1981, Mosley et al, 1985, Cambria et al, 1987, Johnston et al, 1987, Murray et al, 1987). Recently it has been suggested that infusion of a thrombolytic agent into the isolated arterial segment following angioplasty, reduces the rate of early occlusion (Tonneson et al, 1991).

There is now little doubt that successful angioplasty is a cost-effective alternative to surgical treatment (Doubilet & Abrahams, 1984, Jeans et al, 1986), and although the procedure has an overall complication rate of 5%-15% (Al-Kutoubi, 1992), the need for surgical intervention as a consequence of failed angioplasty is only around 3% (Health and Public Policy Committee. American College of Physicians, 1983). The availability of angioplasty has not however reduced the number of patients undergoing surgery for PAOD (Michaels, 1990), partly because the pattern of diffuse disease that usually leads to surgery is the pattern of disease that gives the poorest result in angioplasty (Al-Kutoubi, 1992), although some patients with limb threat considered unsuitable for reconstructive surgery may obtain a satisfactory result from angioplasty (Lu et al, 1982, European Working Group on Critical Limb Ischaemia, 1990).

Percutaneous angioplasty represents a suitable alternative to surgery for selected patients with occlusive arterial disease, but unfortunately the medium to long term results in the femoropopliteal segments (the commonest site of symptomatic disease) are disappointing, with early recurrence of symptomatic stenoses in a large number of these patients (Morin et al, 1986). As a result angioplasty tends to be reserved for the treatment of the claudicant with the discrete stenosis, and the patient with limb-threat who represents a significant operative risk (Al-Kutoubi, 1992).

Attempts to overcome early post-angioplasty restenosis and occlusion have involved the use of **intravascular stents** (DiMassa et al, 1986, Sigwart et al, 1987, Palmaz, 1988), to oppose recoil of elastic vascular stenoses following angioplasty, and to provide a supporting framework for lesions that are liable to dissect or embolise after angioplasty, but these have no clear role in the percutaneous treatment of femoropopliteal lesions (Zollikofer et al, 1991). Additional percutaneous devices such as the flexible **rotating tip catheter** (Kensey et al, 1987) have been devised to liquefy atheroma and recanalise occluded vessels, and although early results have been acceptable (Ginsberg et al, 1988, Vallbracht et al, 1988, Polnitz et al, 1988), the 12 month patency rate is only around 38%. (Triller

et al, 1992). Percutaneous **laser devices** have also been utilised to recanalise occluded vessels (Nordstrom et al, 1988, Leon et al, 1988, Litvack et al, 1988, Michaels, 1990), but the initial enthusiasm for laser recanalisation has not been maintained, as the results appear little better than conventional angioplasty (Bonn 1991, Ginsberg et al, 1985, Sanborn et al, 1988, Nordstrom et al, 1991).

In summary, percutaneous transluminal angioplasty offers an alternative non-operative means of treating selected symptomatic arterial lesions but the long term results of this technique are less satisfactory than those of surgery, particularly in femoral artery disease.

SURGICAL TREATMENT OF PERIPHERAL VASCULAR DISEASE

Development of peripheral vascular surgery

Until the end of the 19th century surgical treatment for peripheral arterial disease was confined to the amputation of gangrenous limbs. The subsequent emergence of techniques of vascular anastomosis (Jassinowsky, 1891, Murphy, 1897, Dörfler, 1899, Carrel, 1902) led to the development of a technique of anastomosis that remains relevant to vascular surgery today (Carrel, 1908), and a number of procedures were performed using vein grafts to bridge arterial defects created by axillary and popliteal aneurysm excision (Lexer, 1907, 1912, 1913), including 2 cases carried out at Glasgow Royal Infirmary (Pringle, 1913).

Further major advances in treatment of occlusive peripheral arterial disease did not take place until vein bypass grafts were introduced in 1951 (Kunlin, 1951). Kunlin and his colleagues described 17 cases of vein bypass of superficial femoral artery occlusions, using autologous saphenous vein in all but one case; 10 grafts were successful, although 3 subsequently occluded in the following 18 months. Freeze-dried arterial homografts were also used to replace occluded segments in iliac and femoral arteries, with similar results (Eastcott, 1953).

With the introduction of prosthetic material for arterial grafts in the 1950's, the range of arterial pathology that could be treated by bypass surgery increased greatly. Initial grafts were manufactured from Vinyl N Cloth (Voorhees et al, 1952), but this has subsequently been followed by Nylon, Teflon (PTFE), and Dacron. Dacron is currently the material of choice for proximal aorto-femoral reconstruction, and patency rates of up to 99% are possible (Reid & Pollock, 1991). Results of femoro-popliteal and femoro-distal grafting are less encouraging, with patency rates of 35-85% being reported (McCollum et al, 1991B), and the

vein bypass graft described by Kunlin (1951), remains the best method of treating femoropopliteal occlusions.

Current status of surgery in peripheral vascular disease

The high patency rates attainable for aorto-femoral reconstruction (Reid & Pollock, 1991), suggest that further major improvements in outcome following such surgery are unlikely. However, surgery for femoropopliteal and infra-popliteal disease has at best a 5 year patency of 80-85% (Mannick et al, 1991, Taylor & Porter, 1991), with significant differences in patency between series from different centres (Tables 1.2, 1.3, 1.4). Infra-inguinal bypass grafts tend to suffer from rapid deterioration in graft patency in the year after surgery, followed by a much lower annual occlusion rate thereafter.

Both graft material and the site of the distal anastomosis have a significant bearing on the outcome of infra-inguinal bypass grafting, with autologous vein giving the best results in all situations, little difference being demonstrated between in-situ and reversed vein (Harris et al, 1987). Prosthetic material, in the form of expanded polytetrafluoroethylene (PTFE) gives similar results to vein above the knee, although the success of PTFE grafts below the knee deteriorates significantly the further distal the anastomosis. In situations where autologous vein is not available, it appears possible to improve the long-term patency of PTFE grafts to calf vessels, by the interposition of a vein collar or patch between the distal end of the graft and the recipient artery (Wolfe & Tyrell, 1991, Cheshire et al, 1992, Taylor et al, 1992).

Indications for surgery

Despite the fact that femoropopliteal disease is the commonest form of occlusive arterial disease (Bloor, 1961), the results of infra-inguinal grafting are such that the operation is often reserved for situations of limb threat, when failure to revascularise the limb would result in tissue loss and ultimately amputation, and for short-distance claudication where the patient is severely impaired by their symptoms. Less severe unilateral, and occasionally bilateral, claudication is a relative indication for surgery, depending on the views of the individual surgeon and the patients' expectations, although few surgeons in the United Kingdom would carry out either below-knee popliteal, or femorocrural grafts, for claudication alone.

Study	*No. cases*	*1 yr. cumulative patency*	*late cumulative patency*	*site popliteal anast.*	*indication*
DeWeese*	70	--	65%(5yr)	A.K.	58% limb
(1977)	43	--	58%(5yr)	B.K.	threat
Hobson (1985)	75	78%	74%(5yr)	n.s.	limb threat
Harris*	102(r.v)#	--	77%(3yr)	n.s.	68% limb
(1987)	98(i.s)#	--	68%(3yr)	n.s.	threat
Leather* (1988)	304	90%	78%(5yr)	B.K.	91% limb threat
Wiseman* (1989)	293	80%	78%(2yr)	n.s.	not specified
Bergamini (1991)	115	82%	64%(4yr)	n.s.	93% limb threat
Kram (1991)	33	85%	74%(5yr)	I.P.S.	limb threat
Taylor	45	88%	88%(5yr)	A.K.	64% limb
(1991)	139	93%	83%(5yr)	B.K.	threat

* indicates that cumulative patency was not defined in terms of primary or secondary patency.

r.v indicates use of reversed saphenous vein, **i.s** indicates use of in-situ vein, where the outcome for the 2 different techniques was specified.

n.s. indicates that the site of distal anastomosis was not specified. (A.K. indicates a proximal popliteal anastomosis, and B.K. a distal popliteal anastomosis. I.P.S. indicates that the anastomosis was to an isolated popliteal segment.).

Table 1.2: Outcome in femoropopliteal vein bypass grafts.

Study	*No. cases*	*1 yr. cumulative patency*	*late cumulative patency*	*site popliteal anast.*	*indication*
PTFE GRAFTS:					
Evans	73	69%	46%(4yr)	A.K.	64% limb
(1981)	25	66%	53%(4yr)	B.K.	threat
Hobson	80	46%(2yr)	22%(5yr)	n.s.	limb
(1985)					threat
Kram	180	89%	55%(5yr)	I.P.S.	limb
(1991)					threat
McCollum*	104	68%	57%(3yr)	n.s.	not
(1991)					specified
Taylor	18	79%	55%(5yr)	A.K.	64% limb
(1991)	18	68%	30%(5yr)	B.K.	threat
Taylor	87	94%	77%(5yr)	A.K.@	72% limb
(1992)	86	88%	65%(5yr)	B.K.@	salvage
HUV GRAFTS:					
McCollum*	87	61%	48%(3yr)	n.s.	not
(1991)					specified
VEIN HOMOGRAFT:					
Dortland*	66	82%	75%(5yr)	A.K.	67% limb
(1991)	39	68%	44%(5yr)	B.K.	threat

* indicates that cumulative patency was not defined in terms of primary or secondary patency.

@ in this series all PTFE grafts had a distal vein patch.

n.s. indicates that the site of distal anastomosis was not specified. (A.K. indicates a proximal popliteal anastomosis, and B.K. a distal popliteal anastomosis. I.P.S. indicates that the anastomosis was to an isolated popliteal segment.).

PTFE: polytetrafluoroethylene.
HUV: Human umbilical vein.

Table 1.3: Outcome in synthetic femoropopliteal bypass grafts.

Study	*No. cases*	*1 yr. cumulative patency*	*late cumulative patency*	*site anast.*	*indication*
VEIN GRAFTS:					
Leather* (1979)	38	--	91%(2yr)	tibio-peroneal	limb salvage
Hobson (1985)	50	45%(2yr)	42%(5yr)	tibial	limb salvage
Leather* (1988)	661	91%	71%(5yr)	tibio-peroneal	91% limb salvage
Bergamini (1991)	246	75%	63%(4yr)	tibial	93% limb salvage
Londrey* (1991)	175	--	63%(5yr)	infra-popliteal	limb salvage
Cheshire* (1992)	89	70%	65%(3yr)	crural	limb salvage
PTFE GRAFTS:					
Hobson (1985)	41	22%	15%(5yr)	tibial	limb salvage
Londrey* (1991)	33	--	28%(5yr)	infra-popliteal	limb salvage
Cheshire (1992)	41	48%	48%(3yr)	crural@	limb salvage
Taylor (1992)	83	74%	54%(5yr)	tibio-peroneal@	72% limb salvage
HOMOGENOUS VEIN GRAFTS:					
Dortland* (1991)	51	71%	41%(5yr)	tibial	86% limb salvage

* indicates that cumulative patency was not defined in terms of primary or secondary patency.
@ in these series all PTFE grafts had either a distal vein collar or vein patch.

PTFE: polytetrafluoroethylene.

Table 1.4: Outcome for femoro-distal bypass grafts (all types).

Graft failure

Factors that have been associated with poor outcome (occlusion) of infra-inguinal bypass grafts are; **1)** undetected proximal disease (Charlesworth et al, 1975, Bergamini et al, 1991), **2)** poor quality of distal outflow (Buda et al, 1976, DeWeese & Rob, 1977), which may be reflected intra-operatively as a low velocity of flow in the graft (Bandyk et al, 1985) or as increased peripheral resistance (Parvin et al, 1985, Brennan et al, 1991), **3)** elevated plasma fibrinogen levels (Harris et al, 1978, Wiseman et al, 1989), **4)** continued cigarette smoking (Greenhalgh et al, 1981, Wiseman et al, 1989), and in particular **5)** the use of prosthetic graft materials (Weisel et al, 1981, Hobson et al, 1985, Rutherford et al, 1988, Londrey et al, 1991).

The use of prosthetic materials for limb salvage procedures in the absence of suitable quality vein however, is still justified, as even with relatively poor results the overall amputation rate in this group of patients can be reduced (Wolfe & Tyrell, 1991), mortality from amputation is higher than that following reconstructive surgery (Ouriel et al, 1988, Cheshire & Wolfe, 1992), and the economic costs of attempting limb salvage, are less than those of adopting a policy of primary amputation for all such patients in whom adequate vein is not available (Cheshire et al, 1992).

Although the outcome of infra-inguinal vein grafting is better than for prosthetic materials, there remains a 1 year failure rate of 7-50% (Tables 1.2, 1.4), and when surgery has been carried out for limb threat, graft failure leads to amputation in many cases (Brewster et al, 1983). Early post-operative failure (up to 30 days) is the cause of up to 25% of graft occlusions, and in both prosthetic and vein grafts is due to a number of factors including poor anastomotic technique, selection of patients with inappropriate severity and pattern of occlusive lesions, systemic hypotension, patient mortality, and intrinsic hypercoagulability (LiCalzi et al, 1981, Rutherford, 1990). Early failure of autogenous vein grafts may also be associated with poor vein quality and injury during the preparation of the vein, particularly injury associated with the use of a valvulotome in the in-situ technique (Donaldson et al, 1992). A number of pre- and intra-operative investigations have been proposed in an attempt to reduce this "technical error" and improve the results of femoro-distal grafting (Parvin et al, 1985, Beard et al, 1989, Bell, 1991, Thompson et al, 1992), but the only technique that is widely available, is that of completion angiography, which should be carried out to assess all femoro-distal grafts following completion of the anastomoses (Gruss, 1989).

Graft occlusions occurring beyond the early post-operative period may be related to some of the factors outlined above, but there are additional features associated with the use of autogenous vein grafts, that are thought to contribute to vein graft occlusion. The belief that vein graft stenoses were related to endothelial injury as a consequence of stripping of venous valves and the application of clamps during surgery, has not been confirmed in prospective studies (Moody et al, 1992), but the process of **neointimal hyperplasia** or intimal thickening (Szilagyi et al, 1973) (Fig. 1.4), the proliferation of smooth muscle and deposition of connective tissue in the intima of the graft, is undoubtedly a major factor in vein graft occlusions (Rutter & Wolfe, 1992). This appears most marked at sites of anastomoses, and may be related to excessive fibrin deposition at these sites (Walton et al, 1985), or to wall tension in the graft causing deformation of the vessel wall (Schwartz et al, 1992). This results in graft stenoses at the sites of intimal thickening which can lead to occlusion in 23-42% of cases, compared with an occlusion rate of 7% in non-stenosed vein grafts (Grigg et al, 1988A, Moody et al, 1989), although up to 65% of strictures may remain asymptomatic.

Late occlusions (more than 2 years post-operatively) of both vein and synthetic grafts are usually a result of progression of atheromatous disease in the native vessels (Rutter & Wolfe, 1992), but atherosclerotic changes can occur in both vein grafts (Walton et al, 1985), and synthetic grafts (Walton et al, 1986), with intramural deposition of lipoprotein and fibrinogen-related antigens contributing to late graft occlusion.

Graft surveillance

The recognition of asymptomatic vein graft stenoses as a precursor of graft occlusion, and the ability to treat stenoses prior to occlusion, with either angioplasty (Berkowitz et al, 1981, Whittemore et al, 1991, Berkowitz et al, 1992), or operative patch angioplasty (Cohen et al, 1986), has emphasised the need for regular surveillance of infra-inguinal vein grafts (Harris, 1992) in the first post-operative year, with a similar, although less intense, surveillance of synthetic grafts (Harris, 1991).

Simple serial ankle-brachial pressure indices were initially thought to be adequate for graft surveillance (Taylor & Fox, 1977, Berkowitz et al, 1981), but subsequent comparison of this with intravenous digital subtraction angiography (DSA) has shown that the use of ABPI alone will fail to detect 50% of stenoses narrowing the graft lumen by more than 50% (Wolfe et al, 1987), although the addition of a "stress test" may improve the accuracy of ABPIs (Wyatt et al, 1990).

Figure 1.4: Neointimal hyperplasia in an in-situ vein graft. This graft occluded 4 months after surgery.

More sophisticated techniques such as DSA are required to detect asymptomatic strictures (Moody et al, 1989, Harris, 1991), but such invasive and expensive techniques cannot be utilised to follow up a vein graft at regular intervals over a period of years.

Duplex scanning offers a less invasive means of visualising both the anatomical and functional (peak systolic velocities and volume flow) arrangement of the graft (Fig. 1.3), and appears to offer an effective means of graft surveillance (Grigg et al, 1988A, Grigg et al, 1988B). An alternative to duplex scanning may be the use of computer-assisted impedance analysis (Wyatt et al, 1991, Davies et al, 1993).

Summary

Surgical treatment of peripheral arterial disease has advanced considerably since Kunlin (1951) first described vein bypass grafting, and excellent results are now obtained with synthetic aorto-femoral grafts. Infra-inguinal grafts continue to have a poorer outcome, with consequent reservations about their use in many situations. A number of factors are responsible for these poor results, including elevated fibrinogen and continued smoking, although more emphasis has been placed on graft material, site of anastomosis, and post-operative vein graft stenosis. In view of the high early occlusion rates of infra-inguinal grafting, graft surveillance is required, but the ideal technique of identifying what are often asymptomatic stenoses has not yet been identified.

PATHOGENESIS OF ATHEROSCLEROSIS

Atherosclerosis is a focal disease of the arterial intima which ultimately leads to stenosis and occlusion of affected vessels. There are 3 classical lesions described: the fatty streak, the fibrous plaque, and the complicated lesion (Ross & Glomset, 1976). The **fatty streak** represents a focal accumulation of intimal smooth-muscle cells containing lipid deposits in the form of cholesterol and cholesterol esters, and is found in up to 50% of the aortic intimal surface by the age of 25 years, regardless of race, sex or environment (Ross & Glomset, 1976). These lesions cause no obstruction to arterial flow, and no clinical symptoms.

The **fibrous plaque** is the typical lesion of advancing atherosclerosis, and its accumulation of lipids and collagen (Barker & Baskerville, 1991) produces a lesion that protrudes into the arterial lumen, although simple fibrous plaques rarely cause clinical symptoms. The relationship between fatty streaks and fibrous plaques

remains unclear, although the former are often found in early life at the same sites as fibrous plaques at older ages (Ross & Glomset, 1976). It has been postulated that some fatty streaks at branches and bifurcations will progress to fibrous plaques (Ross, 1986), while others appear to regress spontaneously (Lindop, 1985). These fibrous plaques are often referred to as atheromatous plaques.

The **complicated lesion** represents a fibrous plaque that has been altered by haemorrhage, calcification, cell necrosis and mural thrombus (Ross & Glomset, 1976), and in these lesions there is typically involvement of all 3 layers of the arterial wall (Schwartz et al, 1989). These complicated lesions are the source of clinical symptoms of occlusive arterial disease. Large plaques can partially or completely occlude the arterial lumen, leading to gradually developing arterial insufficiency. Haemorrhage into the plaque, and/or thrombus formation on the plaque can produce acute arterial occlusion and insufficiency. Ulceration of plaques may be associated with distal embolisation and formation of occlusive thrombi, and thinning of the arterial media in association with plaque formation can lead to aneurysm formation (Lindop, 1985).

Microscopic appearance

Microscopic examination of the lesions of atherosclerosis reveals early accumulation of lipids within smooth muscle cells that are derived from the medial smooth muscle cells. This is accompanied by accumulation of lipids within the connective tissue of the arterial intima, and the proliferation of fibrous connective tissue in the sub-endothelial region. Subsequent necrosis within the deeper layers of the lesion leads to the typical appearance of a structureless accumulation of lipids and cell debris. Lesions are often infiltrated by neutrophils, and lipid-laden macrophages or "foamy cells" (containing cholesterol crystals) are seen within the lipid rich areas of the vessel wall. The process can extend into the media following disruption of the internal elastic lamina, and in all cases the underlying media becomes thin and atrophic (Lindop, 1985).

This represents the usual course of development of atherosclerotic lesions although the lipid component of atherosclerotic plaques seems to be variable, with some occlusive lesions containing relatively little lipid, being more fibroproliferative in nature (Ross et al, 1984).

Aetiology of lesions

Atherosclerotic lesions are presumed to represent the response of arterial intima to injury, as postulated by Virchow, who suggested that atheroma was a

response to vascular "irritation" (Lindop, 1985). The initiating event in the development of atheroma is thought to be endothelial disruption (Ross & Glomset, 1976, Ross, 1986).

The aetiology of this endothelial disruption remains unclear, but a number of apparent "risk factors" have been identified, including hypercholesterolaemia and hyperlipidaemia (Walter & Israel, 1987C, Steinberg et al, 1989), and products of platelet activation (Weiss, 1975, Vermylen et al, 1986, Ross, 1986), which lead to migration and proliferation of smooth muscle cells which lay down an intimal connective-tissue matrix into which plasma-derived lipid is deposited. The process is thought to be halted when endothelial integrity is restored, and the lesions may then regress (Walter & Israel, 1987A). However in situations of repeated or continuous endothelial injury, progressive lesions will develop. Thus platelets and other factors may initiate atheroma, as a response to endothelial injury, and the lesions then grow as a result of smooth muscle proliferation and superimposed thrombus (Walter & Israel, 1987B).

The mechanisms by which elevated plasma lipids and cholesterol levels contribute to atheroma are unclear but may involve alteration of the cholesterol:phospholipid ratio of endothelial cell membranes resulting in endothelial cell retraction at branches and bifurcations (Ross, 1986). Alternatively the oxidation of the low density lipoproteins that accumulate in the arterial wall by macrophage generated free radicals (Carpenter et al, 1991) produces endothelial toxins such as lipid peroxides (Hennig & Chow, 1988). Lipid peroxide levels are elevated in patients with atherosclerosis, and in addition to their toxic effect on vascular endothelium, they appear to increase platelet aggregation (Goto, 1982).

Rheological factors such as altered shear stress at arterial branchings, may also contribute to the development of atherosclerotic lesions and may help to explain the variable distribution of arterial atheromatous lesions (Schwartz et al, 1989).

Cigarette smoking is strongly associated with increased atheroma formation (Powell, 1991). Elevated levels of von Willebrand Factor antigen, which is a marker of endothelial damage, are elevated in smokers, and rise immediately following smoking (Blann, 1992), although the mechanism by which smoking induces endothelial injury is unclear. Smokers also have elevated fibrinogen levels (Meade et al, 1987, Yarnell et al, 1991), and fibrin deposition is seen within atheromatous plaques (Duguid, 1949, Smith & Staples, 1981).

The aetiology of atherosclerosis is therefore multifactorial, with a number of factors contributing to the development of arterial lesions by their effects on

endothelial cells, lipid metabolism, and the mediators of platelet aggregation and thrombosis.

RHEOLOGY AND PERIPHERAL VASCULAR DISEASE

Rheology is the study of flow and deformation of matter and the principles of rheology can be applied to the study of the flow of blood in the arterial tree:

Blood viscosity and red cell aggregation

Viscosity is the intrinsic resistance to flow of a bulk liquid and represents the frictional flow resistance between the theoretical concentric layers that slide telescopically ("shear") over each other in the classical model of laminar flow. This resistance must be overcome by a driving force to cause shearing and subsequent flow (Lowe, 1986), and it falls as increasing temperature reduces the molecular interactions within the fluid that are responsible for it. Blood viscosity is therefore measured at a standard temperature of 37°C (Lowe, 1992).

In situations of laminar flow viscosity is defined as;

$$\text{VISCOSITY (mPa.s)} = \frac{\text{Shear stress (mPa)}}{\text{Shear rate } (s^{-1})}$$

where the **shear stress** is the force applied to a unit area of a fluid layer which causes it to move relative to its adjacent layer, and the **shear rate** is the difference in velocity between two adjacent layers. In situations of laminar flow, the velocity profile is parabolic, with maximal velocity in the central axis of the tube, while shear stress and shear rates are maximal at the vessel wall (Lowe, 1992). From this equation it is apparent that as the viscosity of a liquid increases, a greater shear stress is required to achieve the same shear rate, and hence flow rate (Lowe, 1986).

Blood viscosity is a consequence of frictional interactions between molecules, proteins and cells; **whole blood viscosity** is determined by the **plasma viscosity**, which is related to the concentration, molecular size and asymmetry of plasma molecules (Lowe, 1991), with large molecules such as fibrinogen, contributing more than the smaller molecules such as albumin (despite their lower concentrations), and by the **haematocrit**, and the deformation and aggregation of red blood cells in bulk flow, known as the **relative blood viscosity** (Lowe, 1991).

Blood viscosity is increased at high haematocrit (Lowe et al, 1986) when the ratio of cells to plasma is increased. An indication of red cell deformability is obtained by calculating the relative high-shear blood viscosity, that is, blood viscosity corrected for the effects of haematocrit and plasma viscosity (Lowe, 1992). Red cell deformation is however increased at high haematocrit and plasma viscosity, and increases with higher shear rates as aggregates are disrupted, so that at high shear rates this offsets the tendency of an elevated haematocrit or plasma viscosity to increase whole blood viscosity. High-shear blood viscosity is therefore reported at native haematocrit, and also after correction for haematocrit (**corrected blood viscosity**), and this practice has been adhered to in this thesis, where blood viscosity refers to the haematocrit-corrected blood viscosity, measured at a standard temperature of 37°C.

The flow rate of blood within arteries can be defined in similar terms to the flow of a Newtonian fluid along a rigid tube, using the Hagen-Poiseuille equation;

$$\text{MEAN VELOCITY} = \frac{\text{Pressure gradient} \times \text{tube radius}^4}{8 \times \text{tube length} \times \text{fluid viscosity}}$$

Blood flow in arteries is therefore related to the blood viscosity, in addition to the structural characteristics of the vessels, and the driving pressure. From this equation it is apparent that a 50% decrease in the vessel radius, such as may result from an atherosclerotic plaque, will produce a fourfold decrease in the mean velocity, and in these circumstances, the effect of any changes in blood viscosity will be more marked than in vessels of normal calibre.

In a simple Newtonian fluid such as water or plasma, the fluid viscosity remains constant because the shear rate is directly proportional to the shear stress. Blood on the other hand only displays these Newtonian characteristics at higher shear rates, because at low shear rates, red cell aggregation occurs, leading to increased blood viscosity. These aggregates are dispersed at high shear rates, with the red cells assuming ellipsoid shapes in the centre of the laminar flow, and for shear rates of over 200 mPa.s blood viscosity reaches a constant minimum value (Dormandy, 1981), and high-shear viscosity is the most appropriate measure of blood flow resistance under normal conditions in wide-bore vessels (Lowe, 1991).

Blood Viscosity in Peripheral Arterial Disease

Patients with peripheral arterial disease have elevated blood viscosity at both low and high-shear rates (Dormandy et al, 1973A), and this has prognostic

implications, with a high blood viscosity indicating a more rapid progression of symptomatic occlusive arterial disease (Dormandy et al, 1973B).

Plasma viscosity and haematocrit are also elevated in peripheral arterial disease (Dormandy et al, 1973A, Lowe et al 1986, Ernst & Matrai, 1987), and there is a strong correlation between plasma viscosity and fibrinogen levels in such patients (Dormandy et al, 1973B), with similar correlations observed in population studies (Yarnell et al, 1991). More recently, red cell aggregation has been found to be elevated in patients with peripheral vascular disease (Ernst & Matrai, 1987, Reid, 1991). Although this rise in red cell aggregation could be due to elevated fibrinogen levels bridging erythrocytes to form aggregates (Lowe 1986), this has not been confirmed in clinical studies (Reid, 1991).

Thus plasma viscosity and red cell aggregation would appear to be elevated independently of each other in patients with peripheral arterial disease. The resulting increase in whole blood viscosity could either be an effect of the vascular disease, or a direct causal factor, with a significant aetiological role in PAOD. In the Edinburgh Artery Study the elevation in blood viscosity found in peripheral arterial occlusive disease was related to the severity of the disease, and due partly to increased haematocrit, and partly to increased plasma viscosity and fibrinogen (Lowe et al, 1993).

The rheological changes that have been demonstrated in peripheral arterial occlusive disease may have direct effects on blood flow: an increase in blood viscosity significantly decreases blood flow to limbs and the magnitude of flow changes in response to alterations in blood viscosity are such that a small change in blood viscosity could have significant effects on limb blood flow (Dormandy, 1971), and consequently tissue viability. In the Edinburgh Artery Study, claudication was related to plasma viscosity as well as arterial stenosis (diagnosed by a low ABPI) (Lowe et al, 1993). This observation has implications in graft surgery where flow through the graft is a critical determinant of patency, and in grafts performed for limb salvage this is often only just adequate to maintain patency. Any means of lowering blood viscosity might produce a large enough increase in blood flow to increase the margin of safety, allowing the minor variations in flow that undoubtedly occur in the normal course of events, to pass without flow falling to levels that result in graft occlusion.

WHITE BLOOD CELLS IN PERIPHERAL VASCULAR DISEASE

Neutrophil granulocytes make up between 50 and 70% of the normal white cell population, with around half of their number circulating, and half marginated in the lungs, from where they can be rapidly mobilised in response to certain stimuli. Both the neutrophil count and activation are increased in vascular disease (Jackson et al, 1992) and this may be related to the pathogenesis of occlusive arterial disease.

Rheological effects

Although the absolute number of circulating neutrophils is too small to influence the flow properties of the circulation in larger vessels, the microcirculation is readily influenced by the presence of circulating neutrophils (Lowe, 1986). These large poorly deformable white cells make a significant contribution to microvascular resistance with their ability to block nutritive capillaries (Chien et al, 1987), especially in situations where the perfusion pressure is reduced as a consequence of an upstream occlusion. This results in increased neutrophil transit times through capillaries, with a reduction in flow that enhances neutrophil margination and adhesion to the endothelium (Nash & Shearman, 1992).

Whole blood filterability in peripheral arterial disease is affected by the leucocyte count, with an increased leucocyte count being associated with decreased blood filterability (Alderman et al 1981, Ciuffetti et al 1988), suggesting an increased transit time through the microcirculation. This may lead to local stimulation of neutrophils, resulting in their adhesion to endothelium with release of various mediators that are chemoattractant to other neutrophils. The subsequent recruitment and activation of further neutrophils with their migration through vessel walls may lead to further tissue and endothelial damage (Nash & Shearman, 1992).

In patients with intermittent claudication the absolute neutrophil count and the percentage of activated neutrophils are increased after exercise (Neumann et al, 1990, Hickey et al, 1990), while white blood cells from patients with severe limb ischaemia show impaired filtration through 8μm. and 5μm. pore filters, the filtration properties of the white blood cells returning to normal after amputation of the ischaemic limb (Nash et al, 1988).

It is therefore apparent that neutrophils can have a significant effect on blood flow in the microcirculation, in both their passive and activated forms, and that the rheological effects of these cells may contribute to tissue damage in PAOD.

Consequences of neutrophil activation

Activated neutrophils produce a number of mediators that have the potential to cause significant tissue damage, and the production of activated complement fragment C5a by ischaemic tissue (Bengtson et al, 1987) may be a trigger for neutrophil activation in PAOD (Nash & Shearman, 1992). Tissue ischaemia is present in patients with critical limb ischaemia (Howd et al, 1988), and also occurs following exercise in claudicants (Holdich et al, 1986), and this may lead to leucocyte activation in patients with occlusive arterial disease. Endothelial damage leads to release of platelet activating factor (PAF) which induces platelet aggregation and the platelet release reaction, further contributing to locally increased microvascular permeability and endothelial damage (Anderson, 1985).

Following activation, superoxide anions and proteolytic enzymes are produced by the neutrophil (Lowe, 1990A), and these are capable of inducing endothelial cell damage, leading to the release of arachadonic acid, which is metabolised to thromboxane and prostanoids (via cyclo-oxygenase) and also to leukotrienes (via lipoxygenase). Thromboxanes and leukotrienes are capable of attracting and activating further neutrophils (Nash & Shearman, 1992) thus exacerbating the tissue damage.

Following reperfusion of ischaemic tissue, activated neutrophils transform molecular oxygen into the highly reactive superoxide anion and hydroxyl radical (Weissman et al, 1980). These compounds have the potential to increase microvascular permeability via their toxic effects on endothelial cells, and this increase in microvascular permeability associated with oxygen derived free radicals has been observed following exercise in claudicants (Shearman et al, 1988), although it can be prevented by the prior administration of free radical scavengers (Welbourn et al, 1991).

It has been suggested that both increased white cell numbers and activation are related to smoking habit (Ernst et al, 1987), but there is evidence that some of the association between leucocyte count and arterial disease is independent of smoking (Friedman et al, 1974, Zalokar et al, 1981, Reid, 1991). Although leucocytes may be a marker of smoking habit, their deformability is impaired in response to cigarette smoke (Drost et al, 1992) and they would therefore appear to have an additional role in the pathophysiology of arterial disease.

Studies in patients with ischaemic heart disease suggest that white cell count predicts the risk of myocardial infarction (Friedman et al, 1974, Zalokar et al, 1981), and recurrent coronary events following myocardial infarction (Lowe et al, 1985), while depletion of neutrophil numbers prior to myocardial ischaemia has

been shown to reduce the extent of ischaemic injury in an experimental model (Romson et al, 1983).

Both neutrophil count and activation are increased in patients with an increased risk of vascular disease (Jackson et al, 1992), and a study of 1969 patients with intermittent claudication indicated that an elevated white cell count was a significant predictor of myocardial infarction, stroke and vascular death in these patients (Dormandy & Murray, 1991), while further work has shown that the elevation in white cell count in patients with peripheral vascular disease is related to the severity of occlusive arterial disease, as determined by clinical examination (Reid, 1991).

Whether or not increased numbers of leucocytes are directly contributing to the pathogenesis of occlusive arterial disease, or are merely acting as markers for other, as yet unidentified, aetiological factors, is unclear, and further studies are required.

COAGULATION AND THROMBOSIS IN VASCULAR DISEASE

The recognition that thrombosis is a major component of atherosclerotic arterial disease (Duguid, 1949) has led to a number of studies into the role of the mediators of coagulation and thrombosis. These thrombotic mediators have been studied in patients with various manifestations of atherosclerosis, including peripheral vascular disease, and a number of them have been implicated as risk factors in the development and progression of arterial disease:

Fibrin turnover and fibrinogen

The formation of insoluble fibrin from fibrinogen as a result of the production of thrombin by the coagulation system is fundamental to that system, although there exists a dynamic equilibrium between the processes of coagulation and thrombolysis, including both the formation of fibrin and its' subsequent lysis (Astrup, 1956). The measurement of fibrinogen and of cross-linked fibrin degradation products (FDP's) offers a means of measuring this aspect of the coagulation system in-vivo.

The association between fibrinogen and peripheral arterial disease was demonstrated in 1973 when elevated blood viscosity and fibrinogen levels were reported in patients with occlusive arterial disease (Dormandy et al 1973A). Follow-up studies on this group of patients indicated that a high plasma fibrinogen

level was associated with a poorer prognosis, and likely disease progression (Dormandy et al 1973B).

The Northwick Park Heart Study, a prospective study of the thrombotic component of ischaemic heart disease, showed that major ischaemic cardiac events were increased in men with an elevated plasma fibrinogen level at entry into the study (Meade et al, 1986), and demonstrated a strong correlation between fibrinogen levels and cigarette consumption. It was therefore postulated that the association between smoking and ischaemic heart disease was in part mediated via fibrinogen (Meade et al 1987). The same may well hold true for patients with peripheral vascular disease, and the elevated fibrinogen levels noted by Dormandy (1973A) were a reflection of cigarette consumption in the population studied.

Subsequently fibrinogen has been linked to an increased risk of cardiovascular and cerebrovascular events (Kannel et al, 1987, Yarnell et al, 1991, Qizilbash et al 1991), as well as the prediction of death in patients with stable intermittent claudication (Bannerjee et al, 1992). In addition fibrinogen has emerged as an independent risk factor for stroke and myocardial infarction (Ernst, 1991), while the elevated levels of fibrinogen encountered in occlusive arterial disease and ischaemic heart disease have been shown to be due to more than just the effects of cigarette smoking (Yarnell et al 1991, Lowe et al 1991, Reid 1991).

The Edinburgh Artery Study has shown a strong relationship between the presence of peripheral arterial disease in a population study, and fibrinogen levels (Lowe et al, 1993), confirming the findings of other studies (reviewed by Leng & Fowkes, 1991A). In addition, in a recent study of 157 patients with femoro-popliteal vein grafts, plasma fibrinogen was the strongest predictor of graft occlusion (Wiseman et al 1989), while it was previously reported in a small study that post-operative elevation in fibrinogen levels is of prognostic value (Hamer et al, 1973), although this effect was only observed on subgroup analysis, making interpretation of the results difficult.

The measurement of fibrin degradation products (FDP's) offers a specific means of assessing the level of fibrin turnover, and elevated levels of fibrin degradation products have been found in association with occlusive arterial disease (Al-Zahrani et al, 1992, Peltonen et al, 1992), while the Edinburgh Artery study found a strong inverse correlation between levels of FDP's and the ABPI in a case control study, suggesting a relationship with the severity of the ischaemia (Smith et al, 1993). A similar correlation between FDP's and severity of ischaemia in hospital patients with peripheral arterial disease has also recently been reported (Reid, 1991).

It now seems clear that both fibrinogen and fibrin degradation products are raised in patients with occlusive arterial disease. At least some of the elevation is related to cigarette usage, and may in fact be one of the mechanisms by which smoking exerts its deleterious effects (Meade et al 1987, Yarnell et al 1991, Reid, 1991, Smith et al, 1993). The high levels of fibrinogen seen in PAOD may be a response to the underlying atheroma of the vessel wall, in the manner of an acute phase response (Meade, 1991), however the magnitude of the fall in fibrinogen levels on stopping smoking is too great to be explained by the minimal regression in atheroma that is seen on stopping smoking, and this tends to confirm that a large part of the elevation in fibrinogen is directly related to cigarette consumption.

Pathological evidence of fibrin deposition within atherosclerotic plaques supports the theory that fibrinogen is converted into fibrin within atherosclerotic plaques (Smith & Staples, 1981). It also seems likely that fibrin is capable of binding plasma lipoprotein fractions within plaques, providing an explanation for the high concentration of lipoproteins observed in atheromatous plaques (Thompson & Smith, 1989).

There is in addition to any affect of smoking on fibrinogen levels, a genetic component contributing up to 15% of the variability in fibrinogen levels (Humphries et al, 1987), and a recent case-control study concluded that variation at the ß fibrinogen locus was associated with an increased risk of arterial disease, but that this was not mediated merely by an increase in plasma fibrinogen concentration (Fowkes et al, 1992). Other studies of the genetic contribution to variations in fibrinogen levels between individuals estimate the genetic contribution to be somewhere between 3% (Thomas et al, 1991) and 50% (Hamsten et al, 1987A).

Fibrinogen also has a positive correlation with most other cardiovascular risk factors (Ernst 1991), and the question remains whether fibrinogen has a direct causal relationship with vascular disease, or is merely a marker of the severity of the disease.

There are several mechanisms by which elevated fibrinogen levels may contribute to occlusive arterial disease; fibrinogen links to platelet receptors to promote platelet aggregation (Cook & Ubben, 1990), and platelet aggregability increases with increasing fibrinogen concentrations (Meade et al, 1985). Fibrinogen can promote a hypercoagulable state, leading to deposition of thrombus on atheromatous plaques (Ernst 1991), it may contribute directly to the progress of atheroma via fibrin deposition in the vessel wall (Smith & Staples, 1981, Thompson and Smith, 1989), and it increases blood viscosity by its contribution to plasma viscosity and red cell aggregation (Cook & Ubben, 1990).

It is possible to understand the potential effects of an elevated fibrinogen on the course of PAOD from the above outline, and also to postulate on the likely role of fibrinogen in graft occlusion. The only study reported in the literature that relates elevated fibrinogen levels to subsequent graft failure, reported a strong correlation between plasma fibrinogen (measured 6 months post-operatively) and graft occlusion (Wiseman et al, 1989), as well as between cigarette consumption and graft occlusion. However there is insufficient evidence to support the case for routine estimation of plasma fibrinogen in graft surveillance at the current time. Fibrin degradation products, reflecting fibrin turnover, are perhaps more accurate indicators of on-going thrombosis, and there is some evidence that they may be more useful as predictors of graft occlusion (Reid, 1991).

Von Willebrand Factor (vWF)

Von Willebrand Factor (vWF) is a large multimeric glycoprotein present in platelet α-granules, subendothelium, and plasma (Badimon & Fuster, 1992). It is synthesized by the vascular endothelium (Jaffe et al, 1973, 1974), the major source of plasma vWF (Wagner & Bonfanti, 1991), and also the megakaryocyte (Badimon & Fuster, 1992). vWF has two main functions: it plays a significant role in platelet-vessel wall interactions (Badimon & Fuster, 1992), and acts as a carrier for factor VIII (Meyer et al, 1991). The formation of biologically active multimeric forms of vWF occurs within endothelial cells, which spontaneously release small multimers into plasma and the endothelial basement membrane (to which the vWF becomes bound), although the release of large multimers, stored in Weibel-Palade bodies within the endothelial cells, requires stimulation by inflammatory mediators (Wagner & Bonfanti, 1991). It is these large multimers that are most effective in the promotion of platelet adhesion (Meyer et al, 1991), although all sizes of vWF can carry factor VIII (Wagner & Bonfanti, 1991).

The role of vWF in mediating platelet adhesion involves a conformational change in the vWF molecule, associated with its binding to the subendothelium. Although endothelial cells produce vWF that binds to the subendothelium on secretion, this bound vWF only accounts for 40% of total platelet adhesion, and normal adhesion requires the presence of additional plasma vWF (Sixma & de Groote, 1991). Further studies have indicated that platelet adhesion can be increased beyond that normally observed, in the presence of elevated plasma vWF levels (Zwaginga et al, 1990).

Adhesion of vWF to blood vessel walls is brought about by a number of different collagen binding sites on the vWF molecule. Binding to type I and type

III collagen found in the deeper layers of the vessel wall is mediated by at least 2 separate binding sites (Pareti et al, 1987), and occurs on exposure of this collagen, following vascular injury. Binding to the subendothelium however, involves a separate domain on the vWF molecule, which is thought to bind to subendothelial type IV collagen (Sixma & de Groote, 1991) exposed following endothelial injury.

Following adhesion of vWF to the vessel wall, the molecule undergoes a conformational change that exposes adhesion sites for platelets, which have a number of binding sites for the vWF molecule (Ruggeri et al, 1983). This vWF-mediated adhesion of platelets to the vessel wall requires the presence of calcium, and appears to be of more importance at high shear rates, adhesion at low shear rates being rapidly mediated by proteins such as fibronectin (Sixma & de Groote, 1991). Although vWF-mediated adhesion is much slower than that of fibronectin, it is irreversible, and it is this fact that makes vWF the important mediator of platelet adhesion at high shear rates when platelets are easily washed away from fibronectin.

Platelet vWF is released by various agonists and then binds to the vWF receptors on the platelet membrane. This enhances spreading of the initial contact platelets that have bound to plasma and subendothelial vWF at the site of endothelial injury, and may act as an intercellular bridge between platelets (Gralnick et al, 1991), promoting platelet aggregation at the high shear rates typically encountered in the arterial circulation (Ikeda et al, 1991). The expression of platelet vWF can be inhibited by aspirin, which inhibits 80% of ADP-induced platelet vWF expression, however aspirin has no effect on thrombin-induced platelet vWF expression, although this is inhibited by elevated levels of fibrinogen (Gralnick, et al, 1991).

The in-vitro role of vWF in platelet adhesion at high shear rates appears to be supported by the findings of in-vivo studies where occlusive coronary artery thrombi are produced by a standard injury-stenosis procedure. These occlusive lesions consist of platelet-fibrin thrombi, and only develop in the presence of plasma von Willebrand Factor: The formation of such occlusive thrombi was not observed in von Willebrand Disease pigs deficient in vWF, while restoration of vWF levels to normal resulted in occlusive thrombus formation at the sites of arterial injury. (Brinkhous et al, 1991, Nichols et al, 1991).

The implication of these findings is that a high shear rate, such as that encountered in the arterial circulation, particularly at arterial stenoses, is essential for vWF to produce occlusive arterial thrombi, and for such thrombi to develop the vascular injury must involve exposure of the media (Nichols et al, 1991). The

development of occlusive thrombi can be abolished by the use of a monoclonal antibody to vWF, but nonocclusive microthrombi are still formed suggesting that the role of vWF may be in supporting the progression of these microthrombi to occlusive thrombi (Nichols et al, 1991). In PAOD medial exposure occurs following endothelial injury (Ross & Glomset, 1976), while areas of high shear rate are found at the arterial wall, at sites of atherosclerotic stenosis, and in the microcirculation (Lowe, 1986).

There is evidence that elevation in plasma vWF levels is associated with PAOD (Smith, 1991, Reid, 1991, Blann, 1993). Some of this elevation is related to smoking habit (Blann, 1992), and to other risk factors for atherosclerosis, (Blann et al, 1992, Blann & McCollum, 1992A, 1992B), but some of the elevation of vWF may be a direct consequence of endothelial damage associated with atherosclerosis. Although the mechanism of endothelial damage in atherosclerosis is unclear, there is a correlation between vWF levels and lipid peroxides in cigarette smokers that suggests one possible mediator of endothelial damage is the production of oxygen free radicals (Blann, 1991, Smith et al, 1993).

The precise role of vWF in arterial thrombosis and occlusive arterial disease has yet to be determined despite evidence that this endothelial product is elevated in the disease. This may solely be a reflection of the endothelial damage associated with peripheral arterial disease, but it may be that elevated levels of vWF either lead to an increased risk of developing occlusive thrombi at sites of atheroma, or contribute directly to the development of arterial (or graft) stenosis.

Plasminogen Activator Inhibitor and Tissue Plasminogen Activator.

Thrombin mediated conversion of fibrinogen to cross-linked fibrin is the end result of the coagulation pathway. Subsequent fibrinolysis is brought about by a complex enzyme cascade, that generates localised proteolysis (Kruithof, 1988). Central to this is the action of plasmin, which is converted from plasminogen by the action of endothelial cell-derived tissue plasminogen activator (t.P.A.), and which subsequently cleaves cross-linked fibrin, with the production of FDP's. This fibrinolytic enzyme system contributes to the precise regulation of haemostasis, ensuring that fibrin production is neither excessive (with thrombotic sequelae) nor deficient (with haemorrhagic sequelae). Plasmin activity is specific for excessive fibrin deposits, as t.P.A. is a poor plasminogen activator in the absence of fibrin (Kruithof, 1988).

Plasminogen activator inhibitor (P.A.I.), is a fast-acting inhibitor of t.P.A. found in normal plasma (Kruithof et al, 1984), and released from endothelial cells

and from stimulated platelets (Kruithof et al, 1986), rapidly complexing with t.P.A. to reduce its activity in plasma. The binding of P.A.I. to clot-bound t.P.A. however is much slower (Kruithof et al, 1984), and its main role would appear to be in the regulation of plasma t.P.A. activity.

Although plasma levels of P.A.I. are highly variable in healthy individuals, increased P.A.I. activity has been observed in a number of disease states including cardiovascular and thromboembolic disease (Kruithof et al, 1988). P.A.I. has been shown to be elevated in survivors of myocardial infarction (Hamsten et al, 1985), and independently related to reinfarction (Hamsten et al, 1987B). Elevated pre-operative P.A.I. levels, and reduced t.P.A. levels have also been shown to be associated with post-operative DVT formation in a small series of orthopaedic patients (Paramo et al, 1985).

Some of the wide variation in P.A.I. and t.P.A. levels reported (Kruithof et al, 1988), may be related to the observation that the plasma levels of these substances increase with age (Hashimoto et al, 1987). There is also evidence of a circadian fluctuation in the levels of t.P.A. and P.A.I. (Urano et al, 1990), and in addition, lipid levels and their dietary modification can influence t.P.A. and P.A.I. (Mehrabian et al, 1990, Yamada et al, 1990).

Although there is little data on P.A.I. and t.P.A. levels in peripheral arterial disease, levels of P.A.I. do appear to be elevated in patients with peripheral vascular disease (Speiser et al, 1990, Reid, 1991, Smith et al, personal communication), while both P.A.I. and t.P.A. are elevated in patients with coronary artery disease (Munkvad et al, 1990, Jansson et al, 1991), although this is disputed by others (Meyers et al, 1991).

Elevated levels of P.A.I. observed in peripheral arterial disease may predispose to an impairment of fibrinolysis with an associated thrombotic tendency, which may be of relevance in thrombotic occlusion of both native arteries, and arterial grafts in patients with occlusive arterial disease, however until further studies are carried out this must remain as speculation.

Factor VII

This vitamin K dependent plasma glycoprotein is activated by tissue factor, and is thought to initiate coagulation by the extrinsic pathway, with subsequent activation of factor X. Recent work has however indicated that there are not separate extrinsic and intrinsic pathways, as complex interrelationships exist between the various elements of the coagulation cascade (Messmore, 1981, Prydz, 1992). Nevertheless, population studies have suggested that increased factor VII

levels are associated with an increased risk of ischaemic heart disease (Meade et al, 1986, Broadhurst et al, 1991). This may be related to the ability of Factor VII to initiate the coagulation pathway, with eventual production of fibrin thrombi, and it has been postulated that increased levels of Factor VII lead to increased coagulation (Prydz, 1992).

There are few studies on Factor VII levels in peripheral arterial disease, although an association between acute limb ischaemia or graft occlusion, and reduced factor VII levels has been demonstrated (Cortellaro et al, 1992). Factor VII levels are however affected by a number of non-specific stimuli (Meade, 1991), and the role of factor VII in atherosclerotic vascular disease is not yet well defined.

SUMMARY OF CHAPTER 1

Population studies indicate that the prevalence of symptomatic and asymptomatic peripheral vascular disease in the older community is between 5 and 12.5% in the United Kingdom, and that the incidence of symptomatic peripheral arterial disease rises with age. This is of importance in a society with an increasing elderly population.

Studies of the natural history of symptomatic peripheral vascular disease are reviewed, and indicate that, in addition to a significantly increased cardiovascular mortality rate, between one-quarter and one-third of patients will show a deterioration in their symptoms with time. This highlights the need for careful patient selection prior to surgery for non limb-threatening disease.

Current methods of investigation and treatment of symptomatic peripheral vascular disease have been reviewed, with particular reference to infra-inguinal arterial disease, which represents the majority of symptomatic disease, and in which reconstructive surgery still has a significant failure rate. The role of percutaneous recanalisation procedures is also reviewed.

The known causes of infra-inguinal graft failure are described, and the importance of graft surveillance in the prevention of graft occlusion is discussed.

The pathogenesis of atherosclerosis is briefly reviewed, with reference to the presumed aetiology of the lesions, and current thinking on the role of lipids in arterial disease.

The relationship of blood rheology and thrombotic mediators to vascular disease is reviewed with particular emphasis on blood viscosity, fibrin turnover,

and the endothelial products von Willebrand Factor Antigen, Tissue Plasminogen Activator, and Plasminogen Activator Inhibitor. The possible roles of these variables in the promotion of atherosclerosis, thrombosis, and graft occlusion (and hence their potential value in pre-operative patient selection) are discussed.

Recent evidence linking neutrophil granulocytes and their activation products to intermittent claudication and critical ischaemia are also summarised.

AIMS OF THESIS

This thesis was undertaken to address a number of aspects of blood rheology and levels of potential thrombotic mediators, that have not previously been investigated in patients with PAOD. Namely:

Is there any relationship between the severity of arterial occlusive disease, determined directly by angiography, and the disturbances in blood rheology and levels of potential thrombotic mediators reported to occur in PAOD ? (Chapter 3).

Does the resolution of critical limb ischaemia (either by revascularisation or by amputation) return rheological and thrombotic parameters to the levels observed in an age-matched population ? (Chapter 4).

What are the effects of percutaneous transluminal angioplasty (PTA) on blood rheology and levels of potential thrombotic mediators ? (Chapter 5).

Are pre-operative rheological and haemostatic parameters related to outcome (graft occlusion or death), following infra-inguinal revascularisation surgery, and can the pre-operative determination of these parameters assist in the selection of patients suitable for infra-inguinal revascularisation procedures ? (Chapter 6).

Does the material employed for infra-inguinal revascularisation affect the post-operative levels of potential thrombotic mediators ? (Chapter 6).

These were the main issues that this thesis set out to address, although other related issues that have been investigated are also reported in the relevant chapters.

CHAPTER 2

Materials and methods

Introduction

This chapter details the subjects, materials and methods, and laboratory procedures employed in carrying out the studies undertaken in the course of this thesis.

Patient Selection

All the patients studied were in-patients attending either the Unit for Peripheral Vascular Surgery, Glasgow Royal Infirmary, which is a specialised vascular surgery unit serving the Eastern district of Glasgow, and a tertiary referral centre for surrounding District General Hospitals, or the Level 5 Surgical Unit, Gartnavel General Hospital, Glasgow, where all 3 consultant general surgeons have a special interest in vascular surgery. This unit serves the western district of Glasgow, and also acts as a tertiary referral centre for surrounding District General Hospitals.

A full clinical history and examination were carried out by myself, and the findings recorded for each patient, peripheral pulses were documented, and in 90% of patients, the ankle brachial pressure index (ABPI) was measured prior to blood sampling. Local Ethical Committee approval for patient participation in the studies was obtained, and all patients were given a detailed explanation of the methods and aims of the studies prior to obtaining their consent.

Patients returning for review were seen by myself, in the vascular laboratories of Gartnavel General Hospital and Glasgow Royal Infirmary, where clinical examination and venous blood sampling (as described below), was performed.

The diagnosis of peripheral vascular disease was based on a clinical history of intermittent claudication or ischaemic rest pain, as defined in the report of the Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery/North American Chapter, International Society for Cardiovascular Surgery (1986). In addition, an ankle brachial pressure index (ABPI) of less than 0.90 (Laing & Greenhalgh, 1983), or a fall in ABPI of more than 0.15, following claudication-inducing exercise (Nicolaides, 1991) were required to confirm the diagnosis. 95% of all patients studied also underwent either conventional contrast angiography, or Digital Subtraction Angiography (DSA) as part of their routine assessment.

Critical limb ischaemia, was defined in accordance with the recommendations of the European Working Group on Critical Limb Ischaemia (1990), based on an ankle systolic pressure measurement in the affected limb of

less than or equal to 50 mm Hg, in preference to toe pressure measurement, which is not routinely performed in our vascular laboratories.

Smoking Habit

A detailed smoking history was obtained for all patients, and blood carboxyhaemoglobin levels were measured prior to surgery in all patients, and repeated at each return out-patient visit. Those patients admitting to being current smokers, or in whom the carboxyhaemoglobin levels exceeded 2.0% (Vesey et al, 1982), were classified as current smokers. Patients who had stopped smoking more than 5 years ago were classed as non-smokers, while those patients who had stopped smoking between 1 month and 5 years previously were labelled as recent smokers. Patients in either of these 2 categories in whom blood carboxyhaemoglobin levels exceeded 2.0% at return visits were assumed to have been active smokers for the duration of the studies.

Blood Sampling

A sterile plastic syringe and 19-gauge butterfly needle (Abbott Ireland Ltd. Rep. of Ireland) were used to collect all blood samples. Blood was sampled without venous stasis, after puncturing a full ante-cubital fossa arm vein and then releasing the tourniquet prior to sampling. This method should minimise haemoconcentration and the elevation in tissue plasminogen activator levels associated with the use of a tourniquet (Lewis, 1982, Levin et al, 1984).

Sample Handling

Two 4ml samples of blood for the estimation of full blood count, platelets, and carboxyhaemoglobin were added to tubes containing 0.1ml of liquid dipotassium EDTA 1.5mg/ml (EDTA, Steyne Laboratories), and an additional 5ml of blood for the estimation of haemorheological variables was added to tubes containing dried dipotassium EDTA 1.5mg/ml (Monoject, Sherwood Medical, U.K.). All these tubes were gently mixed and stored at room temperature (20-25°C) for a maximum of 4 hours following collection.

One tube of EDTA blood for full blood count and platelet estimation was sent to the routine laboratory of the Department of Haematology, Glasgow Royal Infirmary, within 6 hours of collection, while a further tube of EDTA blood was sent to the Department of Biochemistry, Glasgow Royal Infirmary, for the measurement of carboxyhaemoglobin levels.

Measurement of whole blood viscosity, haematocrit, and red cell aggregation were performed in the University Department of Medicine, Glasgow Royal Infirmary, within 6 hours of collection, after mixing of the EDTA sample on a rotating and gently oscillating oblique wheel mixer, to prevent phase separation.

After the above measurements, the remaining EDTA-anticoagulated blood was centrifuged at 2,500 g for 10 minutes at room temperature, and 2 samples of 1ml of plasma were aspirated with a plastic Pasteur pipette. One sample was snap-frozen and stored at -70°C in a stoppered plastic tube , while the remaining sample was placed in a stoppered plastic tube until plasma viscosity was measured, usually within 2 hours of separation. Occasionally the specimen was stored at 4°C for up to 48 hours prior to the measurement of plasma viscosity, as such storage has no significant effect on this parameter.

A further 5-10 mls of blood were added to a plain glass tube, and centrifuged at 4°C at 2,500 g for 15 minutes within 4 hours of collection. 2mls of serum was then sent to the Department of Pathological Biochemistry, Glasgow Royal Infirmary for estimation of urea, creatinine, albumin, globulin, and total cholesterol. A further serum sample was stored at -70°C in a stoppered plastic tube.

In addition, 9mls of blood for the measurement of thrombotic mediators were added to a plastic tube containing 1ml of trisodium citrate (0.109M). These tubes were freshly prepared and stored at 4°C, and were transported in a vacuum flask containing an ice-pack at 4°C. After addition of blood they were gently mixed and replaced in the flask at 4°C. The citrated blood was then centrifuged at 4°C for 15 minutes at 2,500 g, within 3 hours of collection, and decanted into small stoppered plastic tubes, each containing 0.5mls of plasma which were snap frozen and stored at -70°C for later assay in batches.

LABORATORY METHODS

Introduction

Except where indicated in the following text, all assays were carried out in the University Department of Medicine Haemostasis and Thrombosis Laboratory, Glasgow Royal Infirmary, by the laboratory scientific staff. The tests were carried out using the laboratory's standard methods, and the laboratory's performance of these tests was satisfactorily assessed, where appropriate, by the National External Quality Control Assurance Scheme (NEQAS). Unless indicated in the text, all

assays were carried out within 6 weeks of specimen collection, on samples that were snap-frozen and stored at -70°C until thawed for assay.

Measurement of Fibrinogen

Clottable fibrinogen was measured on a Coag-A-Mate X2 automated coagulometer (Organon-Technika, Cambridge, U.K.), by the standard Clauss assay, using reagents and standards supplied by Organon-Technika, within 1 week of collection, plasma samples being stored at -70°C prior to assay.

Measurement of Factor VII levels

Factor VII levels were measured by a coagulometric method on an automated coagulometer (ACL 300, Instrumentation Laboratory (UK.) Ltd., Warrington, Cheshire), using reagents supplied by Instrumentation Laboratory (UK.). The ACL 300 measures factor VII levels by performing a modified prothrombin time test: the plasma sample is diluted and added to Factor VII-deficient plasma. Correction of the prolonged clotting time of the Factor VII-deficient plasma is proportional to the concentration (expressed in terms of activity %) of Factor VII in the plasma sample being assayed. The exact activity is then interpolated from a standard calibration curve automatically measured on the instrument.

Measurement of cross-linked FDP levels

The measurement of cross-linked fibrin degradation products was carried out using the "Dimertest" enzyme immunoassay kit, supplied by Agen Biomedical Limited (Agen Ltd., Parsippany, New Jersey, USA).

This kit enables the detection of the degradation products of cross-linked fibrin by the use of an Enzyme-Linked Immunosorbent Assay (ELISA): Initially plasma fibrinogen is converted to fibrin by the action of thrombin, and the resulting fibrin monomers polymerise, forming a soluble gel of fibrin that becomes cross-linked by thrombin-activated factor XIII, forming an insoluble fibrin clot. The fibrinolytic enzyme plasmin cleaves both fibrinogen and fibrin, yielding fibrin degradation products, but only those products from the degradation of cross-linked fibrin contain d-dimer. The "Dimertest" detects those FDP's that contain the d-dimer.

The principle of the test involves the use of a monoclonal antibody, DD-3B6, which recognises d-dimer, and FDP's containing it. The DD-3B6 is bound to microtitre wells, to which either plasma or standard dilutions of D-dimer are

added. The wells are then incubated and subsequently rinsed with buffer solution. Thereafter a Tag monoclonal antibody (DD-4D2), conjugated with horseradish peroxidase is added to the wells, and this binds to cross-linked FDP that has been immobilised by the DD-3B6 antibody bound to the wells. After further washing, addition of substrate leads to the development of colour which is then read by absorbence spectrometry, and compared with the standard curve. Two measurements were made of each sample, and an average value obtained for the specimen.

Elevated levels of cross-linked FDP detected by this method indicate increased fibrinolysis, and imply an alteration in fibrin turnover.

Measurement of von Willebrand Factor Antigen

Von Willebrand Factor antigen (vWF) levels were measured by an ELISA technique (Dako, High Wycombe, UK.), within 6 weeks of specimen collection.

The technique involves the use of microtitre wells coated with a monoclonal antibody to von Willebrand Factor. 100 microlitre aliquots of patients sera are added to some wells, while standards diluted in a buffer solution are added to others. After 1 hour of incubation at room temperature, the plates are washed and peroxide-conjugated detection antibody is added to each well, and incubated for a further hour. After subsequent addition of a colour reagent, the plates are read by absorption photometry at 492 nm. and the results of the patient samples are read against the standard curve plotted for the assay.

Plasminogen Activator Inhibitor (P.A.I.)

Plasma Plasminogen Activator Inhibitor levels were determined by a commercially available chromogenic substrate assay (KabiVitrum Ltd. Uxbridge, Middx., UK.).

The assay involves the addition of a fixed amount of tissue Plasminogen Activator (t.P.A.) in excess to undiluted plasma, where it rapidly forms an inactive complex with the fast inhibitor P.A.I.-1. Plasminogen is then activated to plasmin by the residual t.P.A. in the presence of a stimulator. The amount of plasmin formed is directly proportional to the residual t.P.A. activity, and therefore inversely proportional to the P.A.I. activity in the plasma sample. Plasmin levels are then determined by measuring the amidolytic activity of plasmin on a chromogenic substrate (S-2403), which releases p-nitroaniline, levels of which are determined using a photometer at 405nm. The absorbence of the sample is then

compared with the standard curve generated for each test run, and a value for the level of P.A.I. activity obtained.

Tissue Plasminogen Activator (t.P.A.) levels

Levels of t.P.A. antigen were measured with a commercially available ELISA method (Biopool TintElize, Biopool AB, Umeä, Sweden), utilising a double antibody principle: Plasma samples were added to 2 adjacent wells, one containing normal goat IgG (the "N" well), the other containing goat anti-human t.P.A. IgG (the "A" well), and incubated for 3 hours. Horseradish peroxidase labelled anti-t.P.A. IgG (conjugate) is then added to the wells, and binds to free antigenic determinants on the t.P.A. molecules present in the plasma samples. After incubation any unbound conjugate is washed away, and the amount of peroxidase activity remaining in the wells is determined by the addition of the substrate Orthophenylenediamine Dihydrochloride.

The ensuing colour change is the measured photometrically for each well, and the difference between the "A" and "N" wells calculated. This difference represents the t.P.A. specific part of the response, and the t.P.A. concentration is obtained by plotting this value on the standard curve generated for each test run, standard concentrations of t.P.A. being pre-coated onto each stripwell plate for this purpose.

Urea, creatinine, albumin and globulin

Serum samples for determination of urea, creatinine, albumin, and globulin levels were sent to the routine laboratory of the Department of Pathological Biochemistry, Glasgow Royal Infirmary, where analysis was performed on an Olympus AU 5200 analyser, using reagents supplied by Olympus and Boehringer Mannheim (UK. Ltd.).

Total Cholesterol

Serum cholesterol levels were measured on separated plasma samples, using a Hitachi 717 automatic analyser, and Boehringer Mannheim (UK. Ltd.) reagents, in the University Department of Pathological Biochemistry, Glasgow Royal Infirmary.

Carboxyhaemoglobin Measurement

Carboxyhaemoglobin levels were measured on dipotassium EDTA anticoagulated blood on a Co-Oximeter 282 (Instrumentation Laboratory Ltd.,

Warrington, Cheshire, UK.), within 6 hours of sampling. All samples were processed in the Department of Pathological Biochemistry, Glasgow Royal Infirmary.

Red cell aggregation

Measurement of red cell aggregation was performed by myself and the laboratory scientific staff on dipotassium EDTA anticoagulated samples of whole blood. A photometric technique using an automated Myrenne MA1 Aggregometer (Myrenne GMBH, Roetgen, Germany) was employed. This device measures aggregation in arbitrary units, based on the increasing transmission of light through a sample of 25μl of whole blood over a 5 second period of stasis, prior to which the sample was subjected to a 10 second period of high shear rates of around $600s^{-1}$ to disrupt any aggregates. The greater the red cell aggregation, the greater the transmission of light through the sample, and the higher the numeric value obtained from the aggregometer.

All measurements were performed at room temperature, and at native haematocrit, as standardising for haematocrit appears to make little difference to red cell aggregation in clinical situations (MacRury, 1990). Measurements were repeated twice, and a mean of the 2 readings obtained. If there was a difference of greater than 0.4 units between the 2 readings, then they were repeated again with a fresh sample until a consistent result was obtained.

Haematocrit

Haematocrit was measured by the Hawksley microhaematocrit method (Hawksley & Sons, Lancing, Sussex, UK.) in accordance with the International Committee for Standardisation in Haematology (ICSH) recommendations (1986), and measurements were performed by the laboratory scientific staff and myself:

Duplicate samples of well mixed blood anticoagulated with dipotassium EDTA were drawn up into glass capillary tubes of 1mm diameter, and sealed at one end. The tubes were placed in the Hawksley microcentrifuge and centrifuged at 13,000g for 5 minutes. The haematocrit was then read as a percentage ratio of the red cell pack to that of the whole sample, without correction for plasma trapping. An average of the readings from the 2 samples was taken, rounded to the nearest percentage point.

Plasma and whole blood viscosity

Plasma and whole blood viscosity were measured at 37°C and at high shear rates ($>300s^{-1}$) with a Coulter-Harkness semi-automatic capillary viscometer (Coulter Electronics Ltd., Luton, Beds., UK.). This device measures the time taken for the plasma or whole blood sample to move through a capillary tube using a constant head of pressure, thus enabling calculation of the mean velocity. Viscosity of the liquid can then be calculated from the Hagen-Poiseuille equation (Ch.1, p.52).

From the measurement of whole blood viscosity and the measurement of the haematocrit of the specimen, the haematocrit-corrected blood viscosity was subsequently calculated from a standard formula (Matrai et al, 1987).

Full Blood Count and platelet count

Both the full blood count and platelet count were measured in the Department of Haematology, Glasgow Royal Infirmary, on a Coulter-S Counter (Coulter Electronics Ltd., Luton, Beds., UK.), within 6 hours of the sample being taken.

Ankle Brachial Pressure Index

The ankle brachial pressure indices (ABPI) were measured and calculated in the vascular laboratory of Gartnavel General Hospital, Glasgow, by the laboratory technician, and in the Vascular Laboratory of Glasgow Royal Infirmary, either by a research nurse, or by myself: After a 10 minute period of rest, the brachial, anterior tibial, and posterior tibial systolic blood pressures were measured with a standard sphygmomanometer and an SD1 super Dopplex bi-directional Doppler flow detector with an 8 MHz Doppler probe (Huntleigh Technology plc, Cardiff, UK.). The ABPI was calculated for both limbs by dividing the highest systolic ankle pressure in each limb, by the brachial systolic pressure. The highest absolute systolic pressure for each limb was also documented.

Other vascular laboratory studies employed in this thesis were carried out by myself in conjunction with a vascular research nurse at the vascular laboratory of Glasgow Royal Infirmary, or in conjunction with the vascular laboratory technician at Gartnavel General Hospital, and are documented in detail in the relevant chapters.

Data Storage and Analysis

All data gathered in the course of the work undertaken for this thesis was stored in dBASE III plus files (Ashton-Tate (UK) Ltd., Maidenhead, Berks., UK.) on an IBM-compatible 386SX microcomputer (Dolan Computers Systems Ltd., Carmarthen, Wales), and statistical analyses were carried out by myself using the CSS:Statistica package (Statsoft, Tulsa, USA.), with the exception of multivariate analyses which were carried out by Dr. Janet Love, Research assistant, Robertson Centre for Biostatistics, in conjunction with Dr. G. D. Murray, Director and Reader in Medical Statistics, Robertson Centre for Biostatistics, University of Glasgow. All data plots were created by myself using the CSS:Statistica package.

CHAPTER 3

Blood rheology, thrombotic mediators, and the severity of peripheral arterial disease

INTRODUCTION

Studies in hospital patients with peripheral arterial disease have demonstrated an association between elevated blood viscosity, red cell aggregation, plasma fibrinogen, and peripheral vascular disease (Dormandy, 1971, Dormandy et al, 1973A, Ernst & Matrai, 1987, Reid, 1991), while fibrinogen may have a predictive role in the outcome following reconstructive vascular surgery (Hamer et al, 1978, Wiseman et al, 1989). In addition, population studies have demonstrated that the elevation of plasma fibrinogen and other thrombotic mediators is related to the severity of the disease, as determined by the ankle-brachial pressure index (ABPI) (Leng & Fowkes, 1991A, Lowe et al, 1993, Smith et al, 1993). In spite of being a good screening test (Horrocks & Scott, 1991), the resting ABPI is however an indirect measure of the severity of disease, and gives no clear indication of the extent of arterial lesions. It is also inaccurate in patients who have heavily calcified vessels, such as diabetics, in whom an artificially high ABPI is often found (Krupski & Effeney, 1988, Barnes, 1991).

The aim of this study was therefore to investigate whether or not previously observed associations between blood rheology, potential thrombotic mediators, and the presence of peripheral arterial disease were related to the severity of the occlusive arterial disease when this is determined by the diagnostic standard for the assessment of peripheral vascular disease (Barnes, 1991), namely angiography. The relationship between angiographic extent of occlusive arterial disease and blood rheology and thrombotic mediators, has not previously been studied.

Aims:

1) To compare measures of blood rheology and thrombotic mediators in patients with peripheral arterial disease, and in an age-matched population sample from the West of Scotland.

2) To determine the relationship between the angiographic extent of peripheral arterial disease, red cell aggregation, and blood viscosity, in patients attending a vascular surgery unit for a variety of revascularisation procedures.

3) To determine the relationship between angiographic extent of peripheral arterial disease, and a number of potential thrombotic mediators: plasma fibrinogen, cross-linked fibrin degradation products (FDP), von Willebrand Factor antigen (vWF), factor VII, Plasminogen Activator Inhibitor (P.A.I.), and tissue Plasminogen

Activator (t.P.A.). The relationship between t.P.A. and symptomatic peripheral arterial disease has not previously been reported.

4) To establish the effect of age, sex, and smoking on any association between these variables and the angiographic extent of disease, by means of a multivariate analysis.

MATERIALS AND METHODS

Patients

219 in-patients attending the vascular surgery units at Glasgow Royal Infirmary and Gartnavel General Hospital, had venous blood sampled as previously described, prior to any surgical intervention. These patients were all suffering from intermittent claudication (Ad Hoc Committee on reporting standards, 1986), which had been stable for at least 3 months (n=147), or critical limb ischaemia (n=72) (European Working Group on Critical Limb Ischaemia, 1990).

54 (25%) of the 219 patients had undergone prior revascularisation surgery, and 9 of these 54 had subsequently undergone major amputation (above or below knee). There were an additional 2 patients who had undergone primary major amputation, and 9 patients in whom percutaneous angioplasty had previously been carried out for claudication. Just over one-quarter of the cases (61 patients, 28%) had evidence of tissue infection in one or other limb at the time of venepuncture.

The mean age of the patients studied was 66 years with an age range of 36 to 89 years, and there were 138 males and 81 females.

Control values

The control values for the variables studied were derived from data gathered from 220 subjects aged between 55 and 75 years of age (mean age 65 years), in local population studies (the first, second, and third W.H.O. MONICA surveys). These studies were carried out in random samples of the population aged 25-75 years in the Northern section of the Greater Glasgow Health Board area, to determine the population distribution of cardiovascular risk factors. As part of this study, venous blood samples were taken from all of the subjects. Sample handling and storage were as previously described, and all assays were performed by the staff of the University Haemostasis and Thrombosis Laboratory, Glasgow Royal Infirmary. The results of the assays for 220 subjects aged between 55-75 years

were subsequently retrieved, and provide the age-matched population control values for this study.

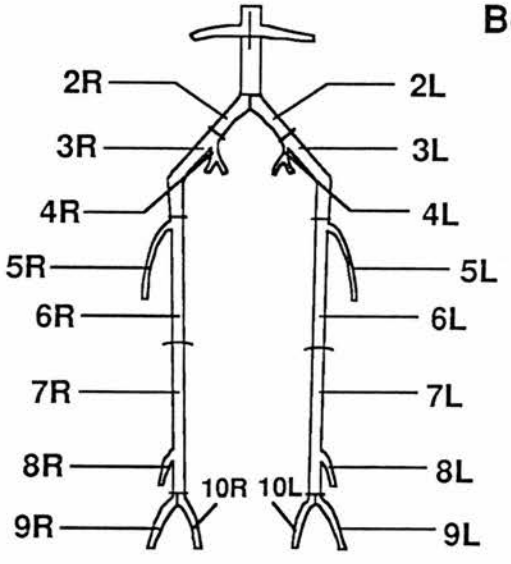
Angiographic scoring

All of the 219 patients studied had undergone angiography between 3 days and 9 months prior to blood sampling, and 193 (88%) had also undergone measurement of the ankle-brachial pressure index (ABPI). Over 90% of the patients had undergone Seldinger arteriography, with retrograde aortic cannulation via the common femoral artery. A small number of patients had undergone translumbar aortography under general anaesthetic, and in 5 patients with infra-inguinal arterial disease, Digital Subtraction Angiography (DSA), was required in addition to obtain adequate imaging of distal run-off.

All of the angiograms were scored by myself, using the scoring system first described by Bollinger and his co-authors (1981). This scoring system divides the arterial tree of the lower limb into a number of segments, and assigns a vectorial score to each segment that is determined by the pattern of occlusions, stenoses, and plaques within it, and an additive score that reflects the severity of the disease within the segment (Fig. 3.1, p.79).

For the purposes of this study, the vectorial component of the angiographic score was ignored, and an additive total for the vascular tree from infra-renal aorta, to 5 cm beyond the origin of each of the 3 calf vessels, was obtained by adding all 19 segmental scores. The greater the overall total, the greater the extent of the vascular pathology. Patent pre-existing vascular grafts were assessed as for native vessels, the underlying by-passed lesions being ignored. For example, if a patient had undergone aorto-iliac grafting for bilateral common iliac disease, the aorta and iliac segmental scores would be derived from the angiographic appearance of the graft and native arteries proximal and distal to the anastomoses, regardless of the appearance of the underlying native common iliac vessels. Patients in whom a prior limb amputation had been performed were assessed on the basis of their remaining vascular tree, and the additive total reflects the extent of arterial disease in the remaining vessels.

The distribution of angiographic scores in the 219 patients approximated to the normal distribution (Figure 3.2, p.80), with a mean value of 106 (S.D. 35), and a range of 26-204. 30 of the 219 angiograms, selected at random, were subsequently rescored by myself, in a blinded fashion, between 12 and 16 weeks after the original scoring assessment had been made. These results were then used to estimate the repeatability of the angiographic scoring system, and as the



Bollinger Angiographic Score

Occlusion	Stenosis>50%	Stenosis<50%	Plaques<25%	LOCATION
	4	2	1	Single
13	5	3	2	Multiple≤H
15	6	4	3	Multiple>H

Occlusive Pattern

Additive Scores

Figure 3.1: Diagrammatic representation of arterial segments and scoring chart for calculating Bollinger angiogram score.

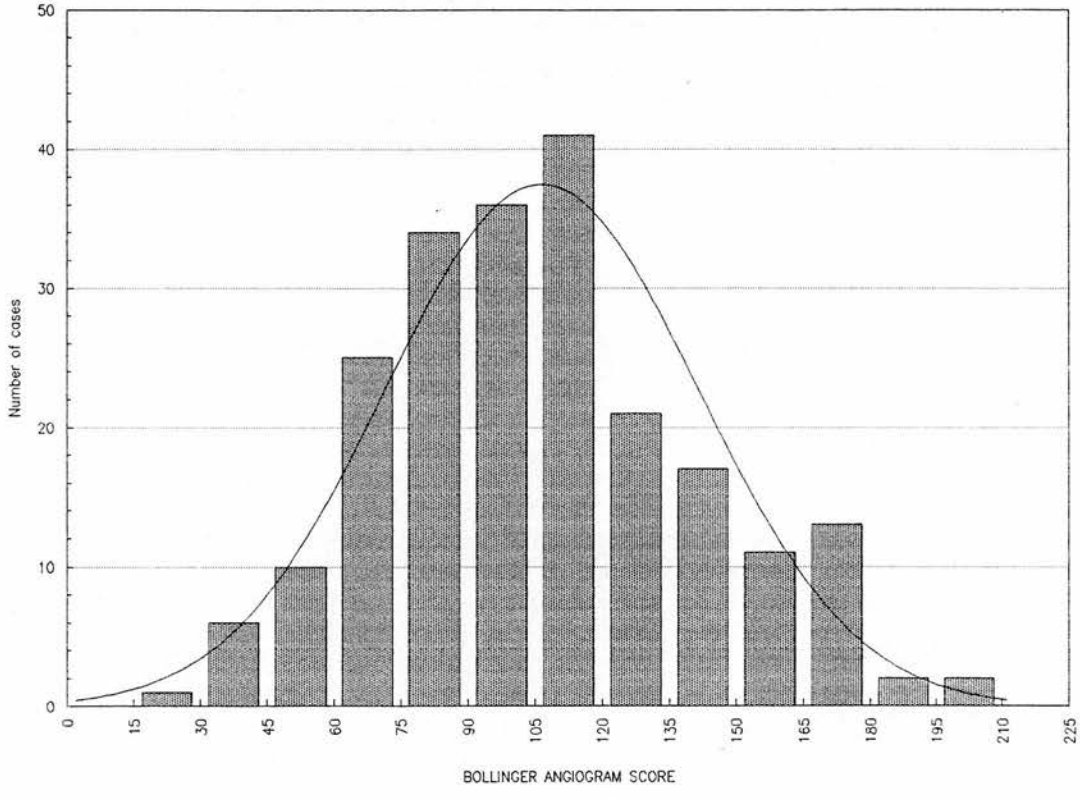


Figure 3.2: Distribution of Bollinger angiogram score in 219 patients with occlusive arterial disease.

Patient	1st score	2nd score	1st-2nd	difference^2
1	71	81	10	100
2	114	104	-10	100
3	83	93	10	100
4	79	88	-9	81
5	153	161	8	64
6	96	98	2	4
7	68	84	16	256
8	100	93	-7	49
9	78	72	-6	36
10	111	122	11	121
11	99	92	-7	49
12	77	76	1	1
13	85	82	-3	9
14	115	121	6	36
15	76	82	6	36
16	152	154	2	4
17	138	140	2	4
18	93	97	4	16
19	91	86	-5	25
20	73	70	-3	9
21	145	151	6	36
22	118	129	11	121
23	101	112	11	121
24	80	99	19	361
25	100	100	0	0
26	67	61	-6	36
27	105	114	9	81
28	54	63	9	81
29	85	87	2	4
30	74	67	-7	49
TOTAL	5860		84	1990
Mean	97.7	2.8		
Total/2n = s^2				33.2
s				5.6

coefficient of variation = 5.6/97.7 = 0.057 or 5.7%

percentage error = 2 x 5.6/97.7 = 0.114 = 11%

Table 3.1: Repeatability of angiogram scores in 30 cases.

calculations (Table 3.1, p.81) show, the coefficient of variation was only 5.7%, giving a percentage error for the measurement of 11%, indicating that the scoring system has an acceptable repeatability in my hands.

Statistical Analyses

With the exception of the multivariate analysis, all statistical analyses were performed by myself as previously described. Multivariate analysis was carried out by Dr Janet Love, Research Assistant, Robertson Centre for Biostatistics, University of Glasgow, in conjunction with Dr. G.D. Murray, Reader in Medical Statistics, and Director, Robertson Centre for Biostatistics.

Multivariate stepwise regression analysis was performed on an IBM-compatible microcomputer, using the BMDP statistical software package (BMDP Statistical Software Inc., Cork, Eire), module 2R (Stepwise regression). Variables entered into the multivariate analysis are listed in table 3.2. For the purposes of the analysis atrial fibrillation, prior ischaemic heart disease, and prior cerebrovascular disease, were combined to give a single category of 'other vascular pathology', and patients were classified as smokers if they had been regular smokers within the past 5 years, and non-smokers if they had stopped smoking before this.

RESULTS

Age, ankle brachial pressure index, and patient characteristics.

Age correlated strongly with the angiographic severity of arterial disease (Table 3.3, p.84, Figure 3.3, p.85) on univariate analysis. There was also a strong negative correlation between the average ABPI (derived from the mean of the highest ABPI reading at each, or the only, ankle), and the angiographic appearance of the peripheral arterial tree (Figure 3.4, p.86). Age and the ankle brachial pressure index were also strongly negatively correlated ($r = -0.32$, $p < 0.005$).

As both the ankle-brachial pressure index and the Bollinger angiogram score are methods of assessing the severity of arterial disease, the ABPI was excluded from the multivariate analysis carried out to determine those factors that were independently related to the Bollinger angiogram score. The presence of critical limb ischaemia was also excluded from the multivariate analysis, as this term also represents a response measure, and it was felt inappropriate to include it when trying to model the underlying disease process.

VARIABLE	DESCRIPTION
Bollinger angiogram score	response variable
sex	male or female
age in years	
other vascular pathology	angina, M.I, T.I.A, C.V.A, or A.F.
diabetes	non-insulin and insulin dependent
hypertension	diagnosed and on treatment
hyperlipidaemia	previously diagnosed
smoking status	current/recent or non-smoker
prior vascular surgery	reconstruction or amputation
Presence of infection	active sepsis in limbs, e.g. ulcer
Antiplatelet therapy	aspirin or dipyridamole
Warfarin therapy	
Haemoglobin	
platelet count	
white cell count	
cholesterol level	fasting prior to surgery
plasma viscosity	
whole blood viscosity	
red cell aggregation	Myrenne aggregation units
corrected blood viscosity	
relative blood viscosity	
haematocrit	
plasma fibrinogen	
cross-linked FDP levels	log(FDP)
von Willebrand Factor antigen	
tissue Plasminogen Activator	
Factor VII activity	
Plasminogen Activator Inhibitor	

Table 3.2: Variables entered into the multivariate analysis to determine the predictors independently related to the angiographic severity of peripheral arterial occlusive disease (Bollinger Score).

VARIABLE	NUMBER	SPEARMAN r VALUE	P VALUE
Mean ABPI	193	- 0.61	$p < 0.0001$
Cross-linked FDP's	213	0.56	$p < 0.0001$
Age	219	0.46	$p < 0.0001$
von Willebrand factor	215	0.40	$p < 0.0001$
Haemoglobin	219	- 0.36	$p < 0.0001$
Haematocrit	208	- 0.32	$p < 0.0001$
Plasma fibrinogen	216	0.30	$p < 0.0001$
Total cholesterol	205	- 0.29	$p < 0.0001$
Platelet count	219	0.25	$p = 0.0004$
Factor VII	200	- 0.21	$p = 0.004$
Plasma viscosity	200	0.18	$p = 0.009$
t.P.A.	195	0.15	$p = 0.04$
White cell count	219	0.12	$p = 0.07$
Corrected blood viscosity	190	0.10	$p = 0.16$
Red cell aggregation	203	- 0.08	$p = 0.24$
Relative blood viscosity	207	- 0.06	$p = 0.38$
P.A.I.	198	- 0.04	$p = 0.56$

Table 3.3: Spearman rank order correlations between angiographic severity of disease (Bollinger angiogram score) and blood rheology and thrombotic mediators, in patients with symptomatic peripheral arterial disease. Results are based on univariate analysis.

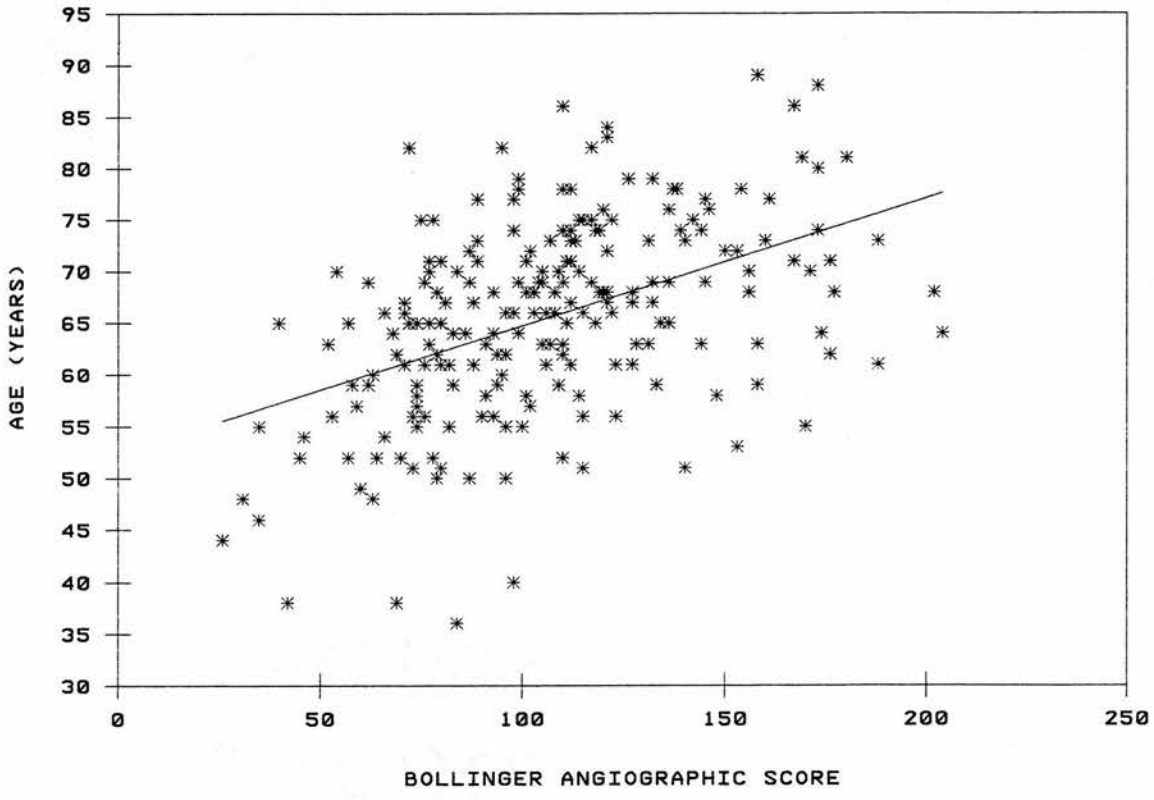


Figure 3.3: Correlation between angiographic severity of disease and age of patient on univariate analysis. ($r = 0.46$, $p < 0.0001$).

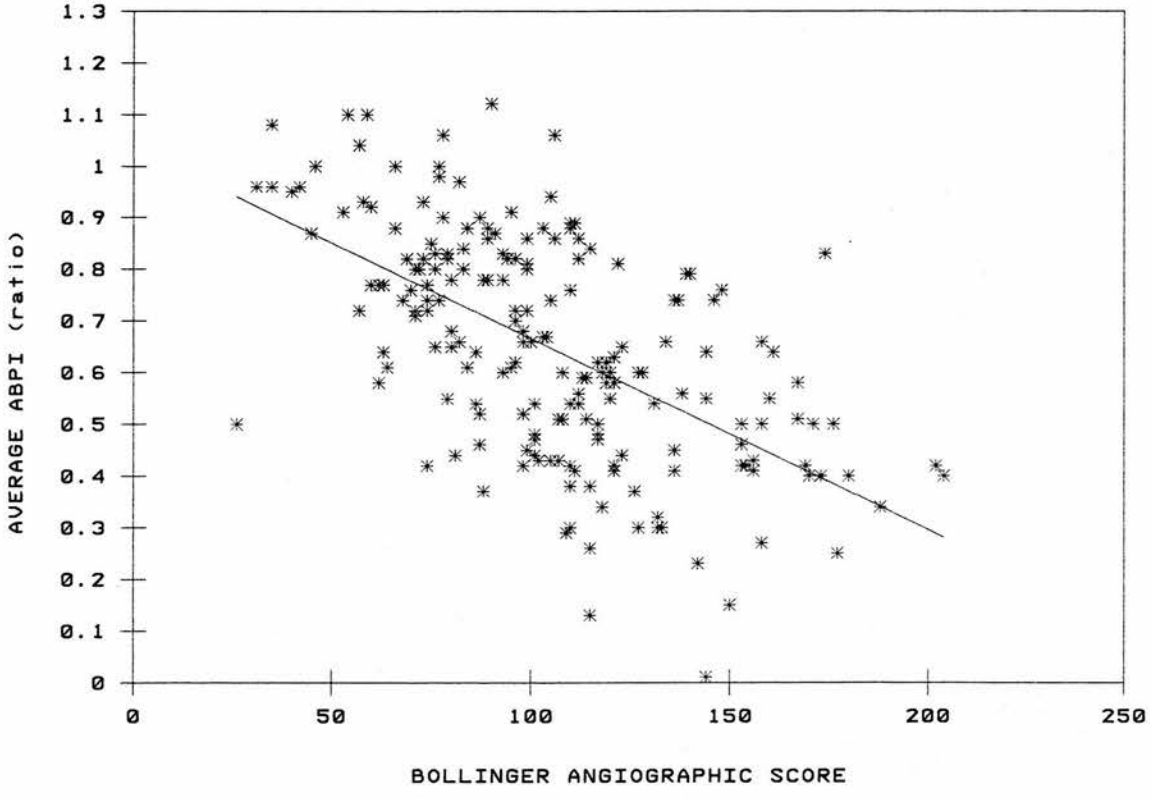


Figure 3.4: Correlation between angiographic severity of disease and ankle-brachial pressure index (ABPI) on univariate analysis. ($r = -0.61$, $p < 0.0001$).

Multivariate analysis of the patient background information indicated that the age of the patient ($F = 21.07$, $p < 0.0001$), prior vascular surgery ($F = 7.51$, $p = 0.007$), the presence of infection ($F = 6.36$, $p = 0.012$), and other vascular pathology (prior transient ischaemic attack or completed stroke, prior myocardial infarction, angina, and atrial fibrillation) ($F = 7.52$, $p = 0.007$), were all independently useful in predicting the Bollinger score of the angiographic severity of peripheral vascular disease. Smoking habit and other factors in the patient history showed no independent association with angiographic severity of disease.

Viscosity and red cell aggregation

Blood viscosity was significantly higher in the age-matched population controls than in patients with occlusive arterial disease (Table 3.4, p.88), while the difference between relative blood viscosity in patients and controls only just reached statistical significance. The differences in plasma viscosity between patients and controls was minimal, and failed to reach statistical significance, while red cell aggregation (measured in Myrenne units), was similar between the two groups, although haematocrit was significantly lower in patients than in controls (Table 3.4, p.88).

On univariate analysis (Table 3.3, p.84) there was a weak positive correlation between the severity of arterial disease, as determined by the Bollinger angiogram score, and plasma viscosity, with viscosity increasing with increasing severity of disease. However there was no apparent association between blood viscosity, or relative blood viscosity, and disease severity, or between red cell aggregation and angiographic severity of disease on univariate analysis.

There was a strong negative correlation between angiographic disease severity, haemoglobin (Figure 3.5, p.89), and haematocrit (Figure 3.6, p.90) on univariate analysis.

Multivariate analysis was performed after adjusting for the significant factors in the patient background (age, prior vascular surgery, infection, and other vascular disease), and deleting cases where results were missing. None of the rheological parameters studied were independently associated with the angiographic severity of peripheral arterial disease in the patients studied, although haematocrit showed a non-significant trend towards association ($p = 0.08$) (Table 3.5, p.91).

Thrombotic mediators

Plasma fibrinogen, vWF, Fibrin Degradation Products (FDP's), and Plasminogen Activator Inhibitor (P.A.I.) were all significantly elevated in patients

TEST	CONTROLS	CASES	MANN WHITNEY U-TEST
W.C.C ($x10^9$/l)	6.2 (5.1-7.9)	8.5 (7.0-10.4)	$p < 0.0001$
Corrected blood viscosity (mPa.s)	3.51 (3.24-3.81)	3.36 (3.14-3.62)	$p < 0.0001$
Fibrinogen (g/l)	2.77 (2.34-3.29)	3.60 (3.00-4.61)	$p < 0.0001$
cross-linked F.D.P. (ng/ml)	94 (62-144)	165 (101-289)	$p < 0.0001$
von Willebrand factor (iu/dl)	114 (85-146)	143 (100-196)	$P < 0.0001$
Plasminogen Activator Inhibitor (% pool)	93 (69-112)	108 (83-157)	$p < 0.0001$
Factor VII (% pool)	113 (93-135)	103 (85-115)	$p = 0.0005$
Haematocrit (%)	44 (41-47)	43 (39-46)	$p = 0.009$
Relative blood viscosity	2.57 (2.39-2.71)	2.50 (2.35-2.68)	$p = 0.05$
Plasma Viscosity (mPa.s)	1.35 (1.29-1.43)	1.34 (1.29-1.41)	$p = 0.16$
Red cell aggregation (units)	4.25 (3.30-5.00)	4.30 (3.40-5.50)	$p = 0.31$
tissue Plasminogen Activator (ng/ml)	8.0 (5.3-11.0)	8.3 (6.2-10.7)	$p = 0.37$
Haemoglobin (g/l)	14.0 (12.0-16.0)	13.6 (12.3-14.9)	n.s

Table 3.4: Levels of blood viscosity and thrombotic mediators in cases and age-matched population controls. Figures are median and interquartile range.

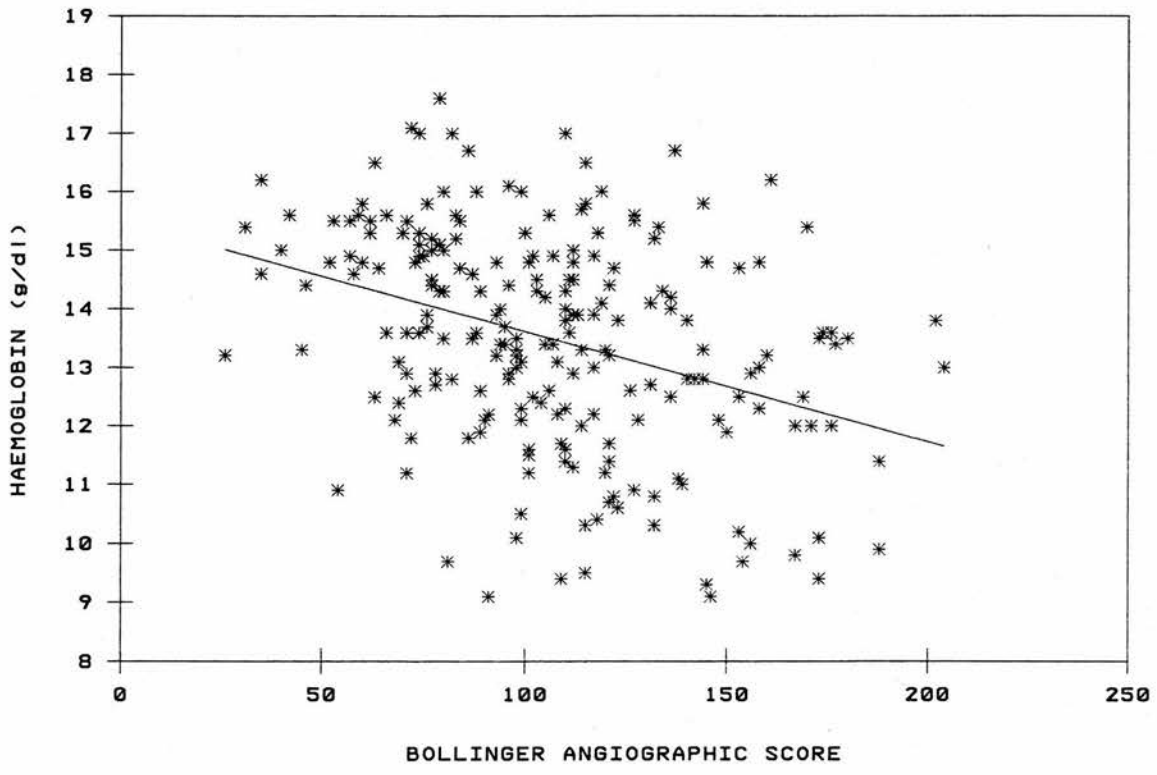


Figure 3.5: Correlation between angiographic severity of disease and haemoglobin on univariate analysis. ($r = -0.36$, $p < 0.0001$).

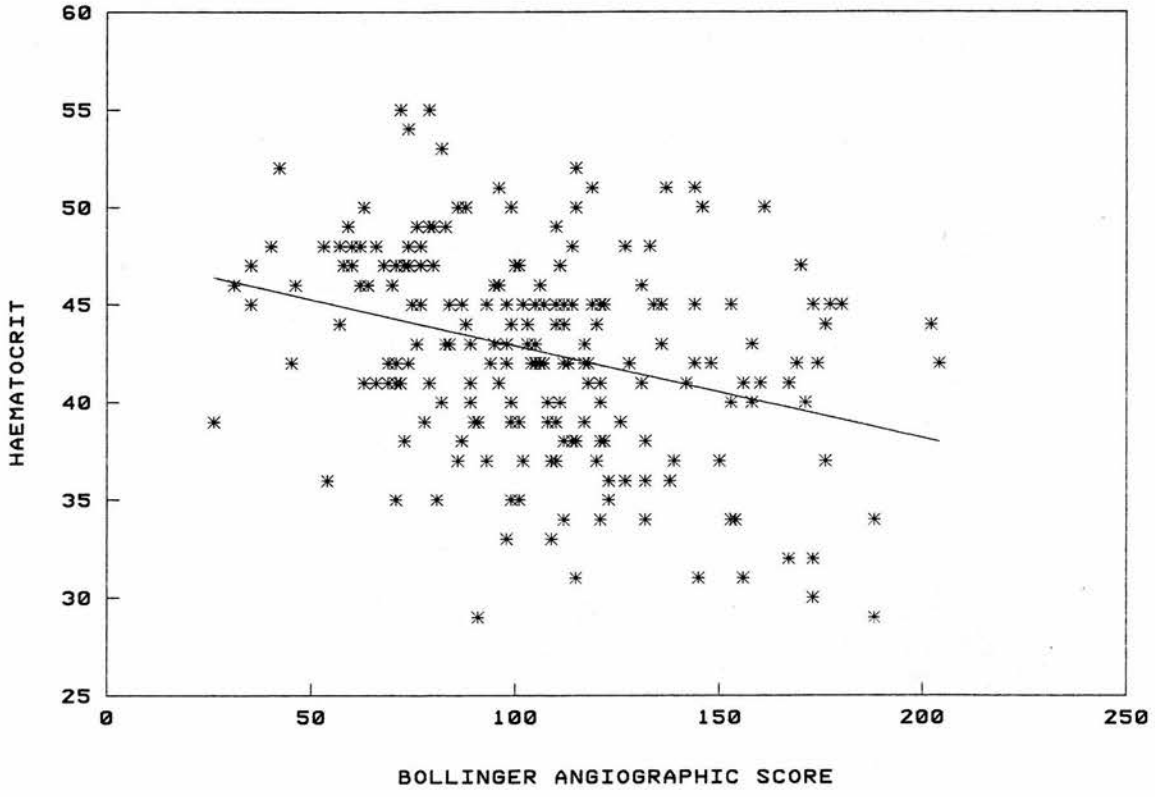


Figure 3.6: Correlation between angiographic severity of disease and haematocrit on univariate analysis. ($r = -0.32$, $p < 0.0001$).

VARIABLE	NUMBER	F-STATISTIC	REFER TO	p VALUE
hyperlipidaemia	213	3.46	F(1,206)	p = 0.06
haematocrit	203	3.01	F(1,196)	p = 0.08
factor VII level	198	2.71	F(1,191)	p = 0.10
platelet count	213	2.66	F(1,206)	p = 0.10
hypertension	213	2.17	F(1,206)	p = 0.14
relative blood viscosity	187	2.10	F(1,180)	p = 0.15
haemoglobin	213	1.76	F(1,206)	p = 0.19
white cell count	213	1.32	F(1,206)	p = 0.25
P.A.I. level	194	1.28	F(1,187)	p = 0.26
corrected blood viscosity	187	1.08	F(1,180)	p = 0.30
warfarin therapy	213	0.85	F(1,206)	p = 0.36
sex	213	0.65	F(1,206)	p = 0.42
smoking	213	0.38	F(1,206)	p = 0.54
plasma viscosity	198	0.18	F(1,191)	p = 0.67
t.P.A. level	190	0.12	F(1,183)	p = 0.73
red cell aggregation	199	0.11	F(1,192)	p = 0.74
fibrinogen	210	0.10	F(1,203)	p = 0.75
von Willebrand Factor	213	0.08	F(1,206)	p = 0.78
diabetes	213	0.03	F(1,206)	p = 0.86
antiplatelet therapy	213	0.00	F(1,206)	p = 1.00
cholesterol level	202	0.00	F(1,195)	p = 1.00

Table 3.5: Relationship between patient characteristics, blood rheology, potential thrombotic mediators, and the angiographic severity of disease, on multivariate analysis after adjusting for the effects of age, infection, prior vascular surgery, other vascular pathology, and log(FDP).

with peripheral arterial disease when compared with the age-matched population controls (Table 3.4, p.88), although tissue plasminogen activator levels were similar in both patients and controls. Factor VII levels were measured in patients not on anticoagulant therapy (n=172), and were significantly lower in patients than in controls.

Univariate analysis revealed strong positive correlations ($p < 0.005$) between angiographic severity of arterial disease, and fibrinogen (Figure 3.7, p.94), von Willebrand Factor (Figure 3.8, p.95), and cross-linked fibrin degradation product (Figure 3.9, p.96), and a weak correlation between tissue plasminogen activator levels and Bollinger angiogram score (Figure 3.10, p.97). There was also a weak negative correlation between factor VII levels and the angiogram score (Figure 3.11, p.98), after exclusion of those patients taking anticoagulant therapy.

Multivariate analysis was carried out after adjustment for significant factors in patient characteristics, and deletion of cases with missing data. Prior to multivariate analysis logarithmic transformation of cross-linked FDP values was performed to normalise the data distribution of this highly skewed variable.

Only cross-linked FDP's (transformed to log(FDP)) were strongly independently associated with the angiographic severity of peripheral arterial disease on multivariate analysis ($p < 0.0001$), although platelet count, plasma fibrinogen, Factor VII, and plasma von Willebrand factor levels, showed a non-significant trend towards independent association with angiographic severity (Table 3.6, p.93). This gave the final model of variables that played an independently significant role in predicting the Bollinger score, and after the inclusion of log(FDP) in the model, other thrombotic mediators did not show any trend towards a significant independent association with the angiogram score (Table 3.5, p.91).

VARIABLE	NUMBER	F-STATISTIC	REFER TO	p VALUE
log (FDP)	213	24.67	F(1,207)	$p < 0.0001$
Platelet count	217	3.55	F(1,211)	$p = 0.06$
Plasma fibrinogen	214	3.32	F(1,208)	$p = 0.07$
Factor VII	202	3.22	F(1,196)	$p = 0.07$
von Willebrand Factor	217	3.14	F(1,211)	$p = 0.08$
t.P.A.	193	1.27	F(1,187)	$p = 0.26$
P.A.I.	198	0.33	F(1,192)	$p = 0.57$

Table 3.6: Relationship between potential thrombotic mediators and angiographic severity of disease on multivariate analysis after adjusting for the effects of significant patient background characteristics (age, infection, prior vascular surgery and other vascular pathology).

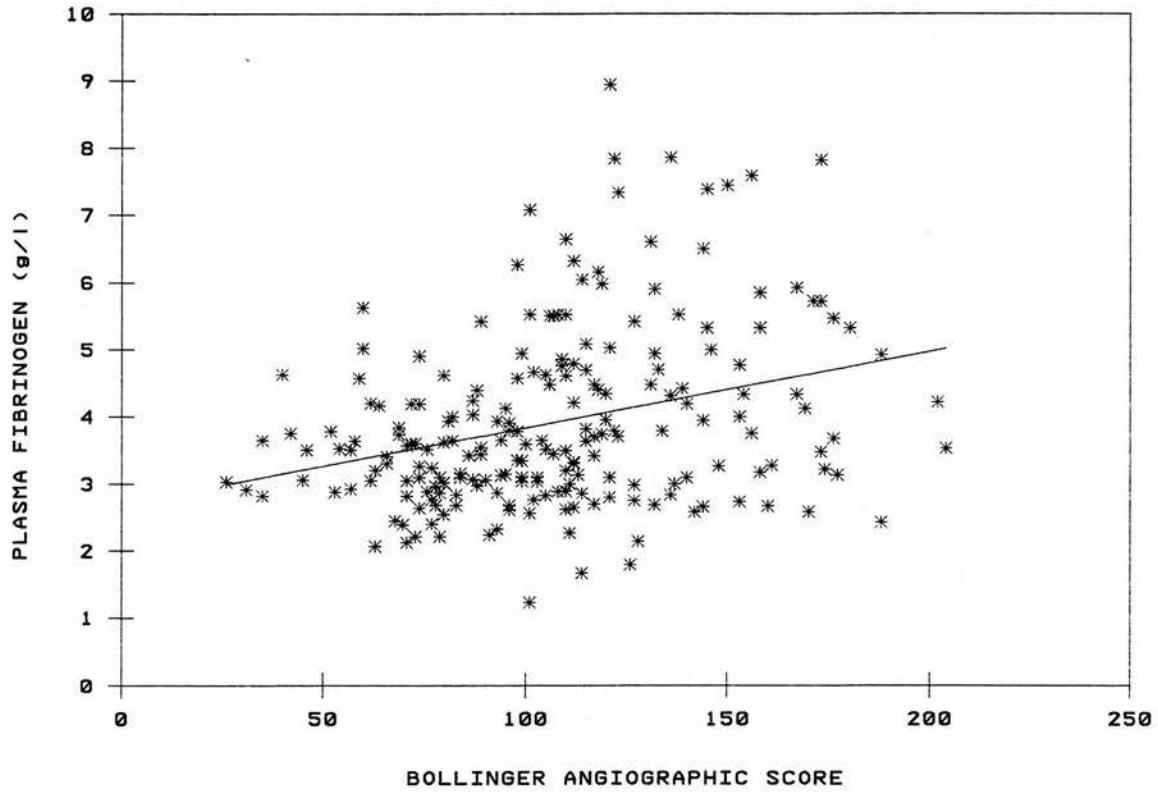


Figure 3.7: Correlation between angiographic severity of disease and plasma fibrinogen on univariate analysis. ($r = 0.30$, $p < 0.0001$).

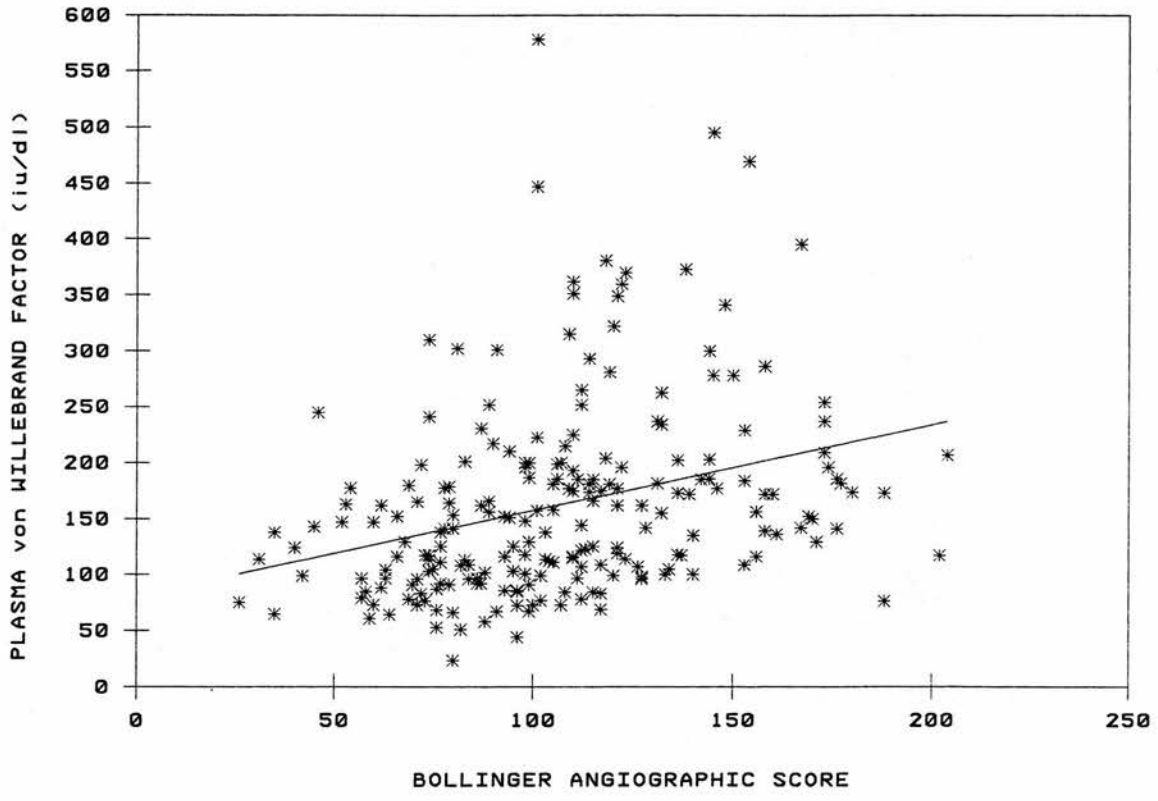


Figure 3.8: Correlation between angiographic severity of disease and plasma von Willebrand Factor (vWF) on univariate analysis. ($r = 0.40$, $p < 0.0001$).

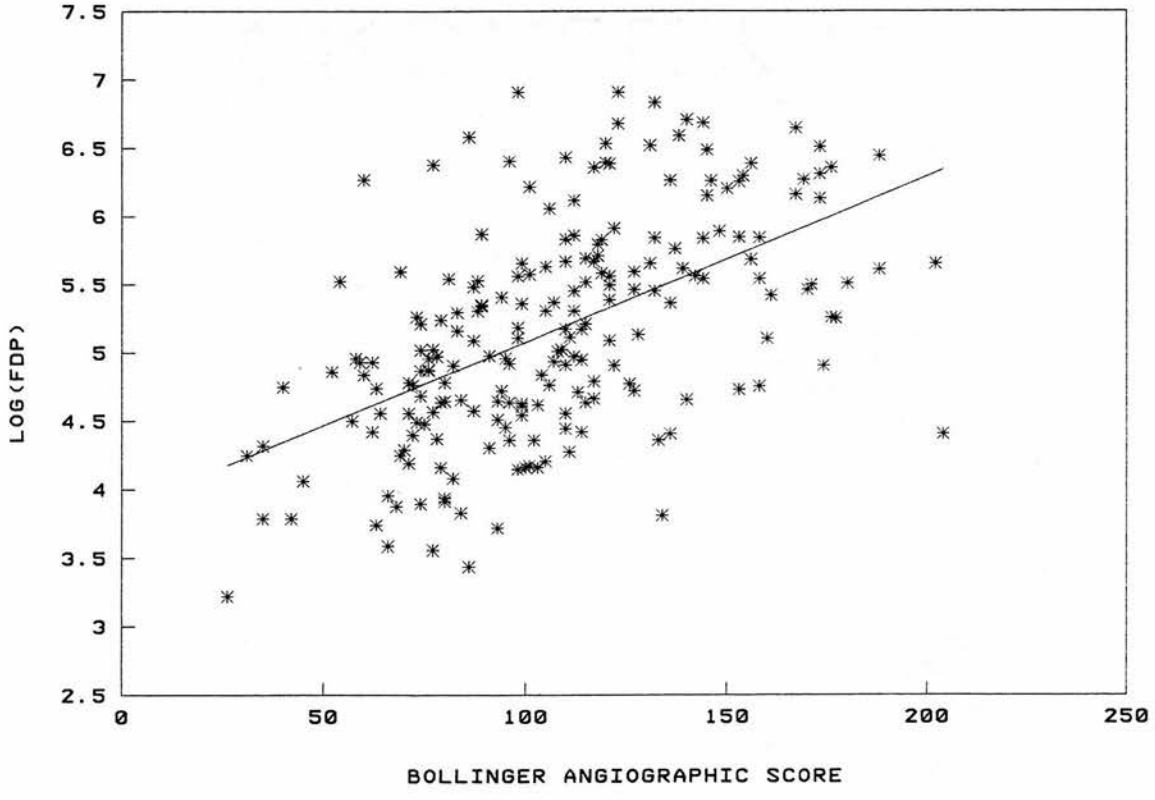


Figure 3.9: Correlation between angiographic severity of disease and cross-linked fibrin degradation products (log(FDP)) on univariate analysis. ($r = 0.56$, $p < 0.0001$).

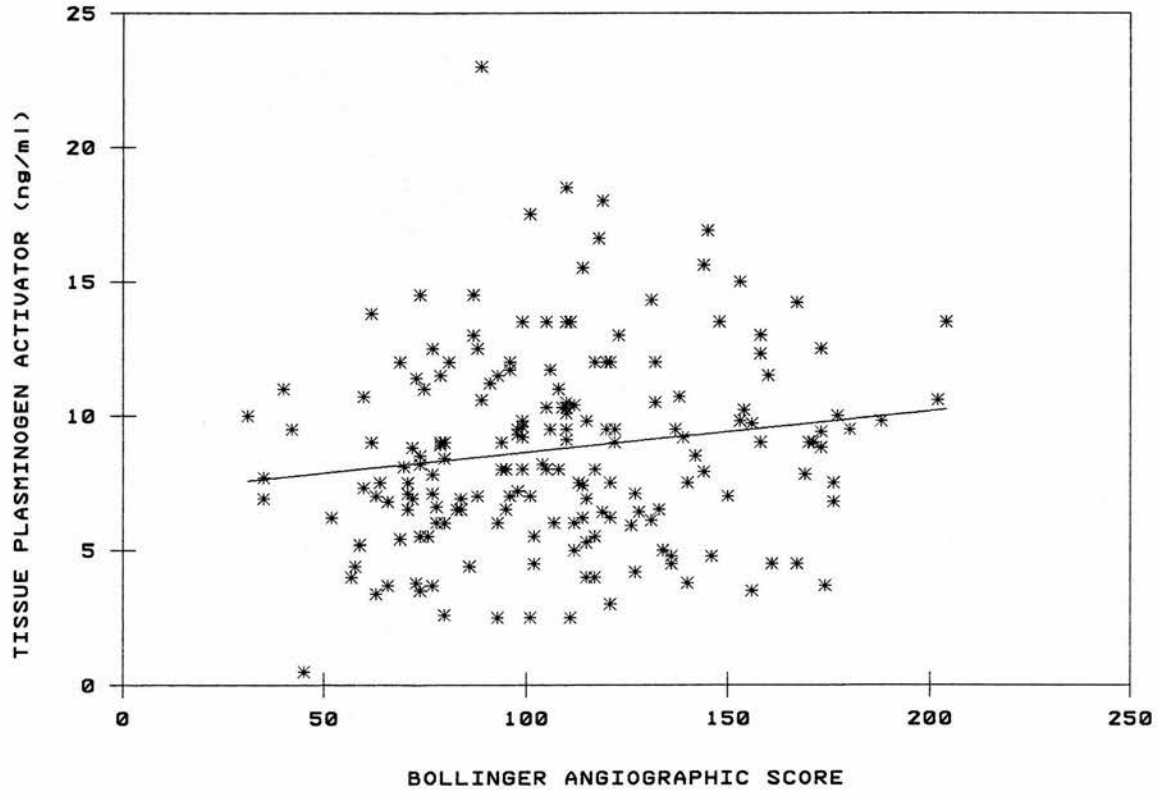


Figure 3.10: Correlation between angiographic severity of disease and tissue plasminogen activator (t.P.A.) on univariate analysis. ($r = 0.15$, $p = 0.04$).

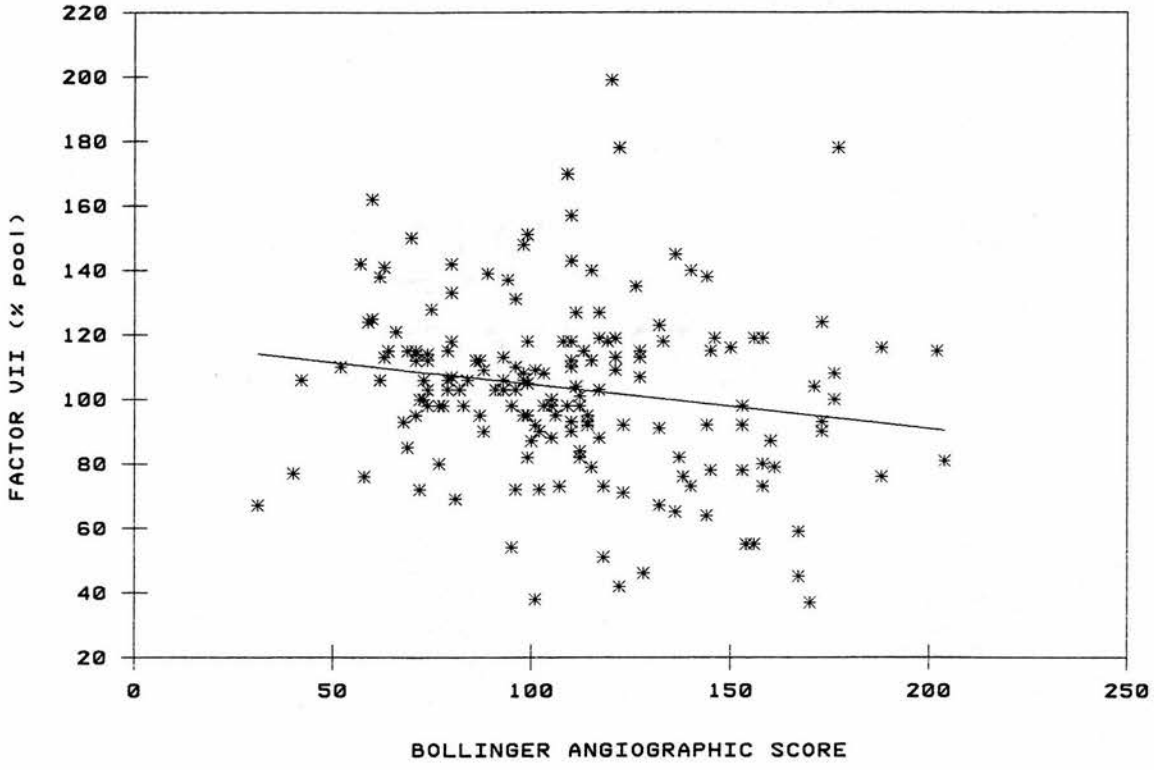


Figure 3.11: Correlation between angiographic severity of disease and factor VII activity on univariate analysis. ($r = -0.21$, $p = 0.004$).

DISCUSSION

The most striking association observed in this study, on both univariate and multivariate analyses, was that between increasing angiographic severity of disease, and increasing age (Figure 3.3, p.85). This finding is in keeping with the results of population studies that have demonstrated an increasing incidence of symptomatic and asymptomatic occlusive arterial disease with increasing age (Bloor, 1961, Kannel et al, 1970, Hughson et al, 1978, Criqui et al, 1985, Fowkes et al, 1991), and confirms that the extent of peripheral arterial disease increases with increasing age.

As fibrinogen, plasma viscosity, and levels of some thrombotic mediators also increase with age (Meade et al, 1979, Lowe et al, 1980), it could be postulated that the association between Bollinger score and blood rheology and thrombotic mediators observed here merely reflect the increased age of patients with the most advanced disease. However comparison of a number of the rheological parameters and levels of potential thrombotic mediators in patients with occlusive arterial disease, with age-matched population controls, reveals significant differences between the two groups, indicating that the altered blood rheology and altered levels of potential thrombotic mediators in PAOD, are due to more than just the effects of increasing age.

Blood viscosity & red cell aggregation in cases & controls

The finding of a small but statistically significant reduction in corrected blood viscosity in cases of peripheral arterial disease when compared with age-matched population controls, appears to contradict previous studies which have demonstrated an increase in blood viscosity in association with peripheral arterial disease (Dormandy et al, 1973A, Lowe et al 1986, Ernst & Matrai, 1987, Lowe et al, 1993). This study also fails to support the observation that plasma viscosity and red cell aggregation are elevated in cases of peripheral arterial disease when compared with controls (Ernst & Matrai, 1987, Reid, 1991). The explanation may be that the present series includes a significant number of patients with critical leg ischaemia, who have low levels of haematocrit and plasma albumin, which leads to a reduction in viscosity and aggregation.

In addition, the results suggest that red cell deformability is increased in cases of PAOD, as manifest by a small reduction in relative blood viscosity when compared with controls. This contradicts previous observations that red cell deformability (measured by filtration) is reduced in patients with peripheral arterial

disease (Reid et al, 1976, Ehrly & Köhler, 1976), although the difference in relative blood viscosity between the two groups studied is of a very small magnitude. This increase in red cell deformability, which accounts for 40% of the inter-individual variation in blood viscosity (Lowe, 1992), may be contributing to the reduction in blood viscosity observed in the study population with occlusive arterial disease.

Some of the aforementioned differences may also be due to the fact that the control population selected in this study consisted of an age-matched random population control, rather than the 'normal' controls (selected on the basis of a negative history of smoking and any cardiac or vascular disease) used in other studies, where the control values for plasma and whole blood viscosity are slightly lower than were seen in the control population used here (Lowe et al, 1993). Thus the median control value in this study reflects blood viscosity in an unfiltered population which will contain cases of peripheral arterial disease, amongst other pathologies affecting blood viscosity, as well as being drawn from a population 5 to 10 years older than control populations in other studies (Lowe et al, 1986, Reid, 1991). The observation that the values obtained for plasma viscosity and red cell aggregation in patients in this study are similar to those from other studies (Reid, 1991), tends to confirm the belief that the results obtained in these studies reflect a different policy in control selection.

Blood viscosity, red cell aggregation, & disease severity

Despite significant univariate correlations between blood and plasma viscosity, and the angiographic severity of arterial disease, multivariate analysis failed to demonstrate any independent association with disease severity. This indicates that the elevation in plasma viscosity and reduction in blood viscosity associated with peripheral arterial disease are consequent upon other features that accompany PAOD: the correlation between angiographic severity of disease and plasma viscosity being due to the effect of age (Lowe et al, 1980, Reid, 1991), and the presence of infection, which was independently predictive of disease severity.

The strong negative correlation between disease severity, haemoglobin, and haematocrit, on univariate analysis, and the trend towards a significant independent association between haematocrit and Bollinger score on multivariate analysis, is in contrast to the findings of most studies in patients with peripheral arterial disease, which associate occlusive arterial disease with increased haematocrit (Lowe et al, 1986).

The association between low haemoglobin and severe arterial disease may be similar to the "haematological stress syndrome" reported in patients with PAOD (Stuart et al, 1981). This syndrome is found in the blood of patients with chronic inflammatory disease states, and consists of anaemia, platelet activation and hyperfibrinogenaemia amongst other features. The observation that this response to chronic inflammatory conditions occurs in occlusive arterial disease, suggests similarities to these conditions and may indicate that other responses associated with chronic inflammatory disorders, such as the anaemia of chronic disease (Rowan, 1985), also occur. This would account for the lower haematocrit observed in cases of arterial disease, and it is likely that the observed negative correlation between haemoglobin level and disease severity reflects the presence of the anaemia of chronic disease in patients with PAOD, in the same way that the correlation between plasma fibrinogen and disease severity reflects the haematological stress syndrome. However, as the multivariate analysis indicates, the associations between haemoglobin, haematocrit, and angiographic severity of disease, are not independent of the presence of infection and prior vascular surgery. This suggests that the haematological stress syndrome in severe PAOD may be a consequence of associated chronic low-grade infection, and perhaps the presence of synthetic vascular prosthesis, although further studies are required to determine whether or not this is the case.

Thrombotic mediators in cases & controls

This study has confirmed the findings of previous studies that demonstrate an increase in potential thrombotic mediators in the presence of peripheral arterial disease (Dormandy et al, 1973A, Reid, 1991, Lowe et al, 1993): Plasma fibrinogen, cross-linked FDP's, and von Willebrand Factor have all been shown to be elevated in cases of peripheral arterial disease, when compared with disease-free controls (Reid, 1991), although one cause of these alterations may be attributed to smoking habit (Smith et al, 1993).

The relationship between levels of tissue plasminogen activator (t.P.A.) and occlusive arterial disease have not previously been reported, although levels of plasminogen activator inhibitor (P.A.I.) and Factor VII have been found to be elevated in a small number of patients with peripheral vascular disease (Reid, 1991), and increasing age is known to increase levels of both t.P.A. and P.A.I. (Hashimoto et al, 1987). The results in this study indicate that levels of t.P.A. in patients with occlusive arterial disease are similar to those found in an age-matched population sample, although levels of P.A.I. are significantly greater. This

elevation in P.A.I. levels in patients with occlusive arterial disease may shift the fibrinolytic balance towards the inhibition of fibrinolysis, with a corresponding increase in intravascular thrombus formation leading to vascular occlusion, and an increased likelihood of thrombus being incorporated into plaques (Duguid, 1946). It would also be in keeping with the impaired fibrinolysis demonstrated in patients with other manifestations of vascular disease, such as ischaemic heart disease (Sakata et al, 1990, Hamsten & Wiman, 1992). The elevated levels of cross-linked FDP's observed in PAOD, indicating increased lysis of cross-linked fibrin, tend however to contradict this belief, although the mechanisms that regulate thrombosis and thrombolysis are of such complexity that the effect of an alteration in the levels of a single component cannot be realistically predicted.

The finding of a reduction in Factor VII activity in patients with occlusive arterial disease contradicts a previous study which suggested that Factor VII levels were increased in the presence of vascular disease (Reid, 1991). The control population in this current study however, show levels of factor VII activity comparable with those observed in the cases reported by Reid (1991), suggesting that age-related elevations in Factor VII activity (Folsom et al, 1991) may have been responsible for the alteration in Factor VII levels reported in that study. This belief is supported by the absence of age-matched controls in the study.

Low levels of Factor VII activity have been found in patients with peripheral arterial disease in other studies (Cortellaro et al, 1992), and the low factor VII levels observed here may be the consequence of an acute inflammatory response (Meade, 1991) to limb sepsis, which was present in many of the cases studied.

Thrombotic mediators & disease severity

The results of univariate analysis in this study provide further evidence that fibrinogen, FDP's, and vWF increase with increasing severity of arterial disease, as determined by angiography rather than indirect methods. These correlations, which have been observed in other similar studies (Reid, 1991, Lowe et al, 1993), raise the question of whether or not the observed associations between potential thrombotic mediators and peripheral arterial disease are causal, or merely represent a haematological response to the underlying chronic disease process (Reizenstein, 1979).

Multivariate analysis suggests that cross-linked FDP levels are independently associated with the angiographic severity of occlusive arterial

disease, while there is only a trend towards independent association for plasma fibrinogen, von Willebrand Factor, and Factor VII levels, which disappears when log(FDP) is taken into account. In terms of predicting the angiographic severity of occlusive arterial disease, log(FDP) is therefore the most useful potential thrombotic mediator. As multivariate analysis indicates, the association between increasing log(FDP) and increasing severity of occlusive arterial disease is independent of smoking habit, infection, age, other cardiovascular pathology, and also prior vascular surgery. This is in keeping with other studies where the elevated FDP level in peripheral arterial disease has been shown to be independent of other vascular risk factors (Speiser et al, 1989, Reid, 1991).

Elevated levels of cross-linked FDP's may reflect increased turnover of fibrin in PAOD, perhaps as a consequence of formation of superadded thrombus on atherosclerotic plaques (Lindop, 1985). In this way FDP's may be merely an indicator of the severity of the arterial disease; increased thrombus formation being more likely in the presence of widespread arterial disease, where there are a greater number of atherosclerotic plaques. Alternatively the presence of increased levels of cross-linked FDP's may lead directly to a worsening of disease severity:

There is evidence from pathological studies that there is a continuous turnover of cross-linked fibrin within the arterial intima (Smith et al, 1990), and that FDP's are usually found in the advanced lesions of atherosclerosis (Bini et al, 1989). In addition it has been suggested that low molecular weight FDP's stimulate the release of growth factors from the endothelium (Lorenzet et al, 1992), while other fragments from the breakdown of cross-linked fibrin are thought to have mitogenic properties (Thompson et al, 1990).

While these findings derive from animal work, and some concern fibrin degradation products other than the d-dimer measured in this study, all these stimulatory FDP's are produced from the degradation of cross-linked fibrin, along with d-dimer fragments, and will be present in similarly increased concentrations. It is therefore feasible that the raised FDP's encountered in patients with occlusive arterial disease contribute to the acceleration of the disease by stimulating the focal smooth muscle cell proliferation that is a major component of atherosclerosis (Ross et al, 1984, Ross, 1986). Some FDP's are capable of increasing vascular permeability and inducing retraction and disorganisation of endothelium (Loscalzo, 1992), which may account for the close relationship between FDP levels and von Willebrand Factor levels, which may be reflecting the endothelial damage induced by FDP's.

No other thrombotic mediators show an independent association with the angiographic severity of peripheral arterial disease, suggesting that any associations between occlusive arterial disease and these potential thrombotic mediators are a consequence of the increased incidence of infection and other cardiovascular pathology found in patients with the most severe disease, and in the case of t.P.A., the increasing age of the patients (Hashimoto et al, 1987). Plasma fibrinogen is known to be elevated in acute inflammation (Meade, 1991), and will therefore be elevated in the presence of limb sepsis. Von Willebrand Factor levels are also increased by a number of non-specific stimuli (Ingerslev, 1990), including chronic inflammatory conditions (Blann et al, 1992), while Factor VII levels fall in acute and chronic illness (Meade, 1991).

Despite the lack of any independent association between these potential thrombotic mediators and the severity of arterial disease, some of them may well play a role in peripheral arterial disease, either indirectly by increasing thrombin formation and FDP levels, or by directly contributing to the progression of peripheral atherosclerosis. Increasing levels of plasma fibrinogen may promote increased formation of fibrin thrombi, with subsequent occlusion of vessels and a corresponding increase in the angiographic severity of the disease.

Increased levels of von Willebrand Factor, which is an essential requirement for the development of occlusive thrombi at sites of arterial injury (Brinkhous et al, 1991), may lead to increased formation of intravascular thrombi, with subsequent development of atherosclerotic plaques (Duguid, 1946). Animal studies have suggested that absence of plasma von Willebrand factor confers resistance to atherosclerosis (Badimon & Fuster, 1992), and it may follow that elevated vWf levels are associated with accelerated development of atherosclerosis, as an increase in plasma vWF can lead to increased platelet adhesion and aggregation (Zwaginga et al, 1990).

Thus, even if the correlations between potential thrombotic mediators and the angiographic severity of disease are a consequence of other conditions commonly associated with peripheral vascular disease, the subsequent alterations in fibrinogen and von Willebrand Factor levels may contribute to the disease process. The effect of reversing the disease process by surgical intervention or angioplasty is studied in subsequent chapters.

Summary

These findings confirm previous observations that plasma fibrinogen, cross-linked FDP's, von Willebrand Factor, and P.A.I. levels are all elevated in patients with peripheral arterial disease, and indicate that the degree of elevation in these thrombotic mediators correlates with the angiographic severity of the arterial disease, although only the level of cross-linked FDP's is independently predictive of the severity of the disease. Elevations in all these thrombotic mediators may either be consequences of peripheral arterial disease, or may be of aetiological importance, as a result of their effects on thrombus formation and fibrin turnover, and the observations support the belief that there is an increase in fibrin turnover with increasing severity of peripheral arterial disease.

The results also suggest that factor VII levels are reduced in peripheral arterial disease, in contrast to previously reported findings. This is likely to be a result of acute and chronic inflammatory responses to peripheral vascular disease.

These studies have determined levels of tissue Plasminogen Activator (t.P.A.) in patients with symptomatic peripheral arterial disease. This has not previously been reported, however the results failed to show any significant elevation in t.P.A. levels in association with arterial disease, and failed to demonstrate any relationship between t.P.A. and the angiographic severity of peripheral arterial disease.

Previously reported elevations in plasma and blood viscosity in patients with peripheral vascular disease were not confirmed by these studies, with a reduction in blood viscosity, haemoglobin, and haematocrit being observed in contrast. This may be a result of a haematological stress response to chronic inflammatory processes resulting from severe peripheral vascular disease.

The observations made confirm that peripheral vascular disease is associated with marked systemic changes in blood rheology and levels of potential thrombotic mediators. Subsequent chapters of this thesis will investigate the effects on blood rheology and potential thrombotic mediators, of reducing the severity of peripheral arterial disease by surgical intervention.

CHAPTER 4

The effect of revascularisation surgery on blood rheology and thrombotic mediators in critical limb ischaemia.

INTRODUCTION

In the work reported in the previous chapter it was observed that haematocrit-corrected blood viscosity and relative blood viscosity, together with the levels of a number of potential thrombotic mediators (plasma fibrinogen, cross-linked fibrin degradation products, von Willebrand factor, factor VII, and plasminogen activator inhibitor (P.A.I.)) were altered in patients with peripheral arterial occlusive disease, and that these alterations were more marked in patients with angiographic evidence of severe disease. These alterations in potential thrombotic mediators may act via a number of pathophysiological mechanisms to further both the development of atherosclerosis and arterial occlusion (Bini et al, 1989, Zwaginga et al, 1990, Badimon & Fuster, 1992), leading to the development of critical limb ischaemia.

If these alterations in potential thrombotic mediators occur solely as a consequence of biochemical derangement secondary to tissue ischaemia, then it is likely that successful revascularisation or amputation will reverse these potentially harmful alterations in blood rheology and levels of thrombotic mediators. In order to investigate this possibility, patients with severe peripheral arterial occlusive disease, meeting the definition of critical limb ischaemia (European Consensus Document, 1989) have been studied, both before and after surgical intervention to resolve their critical limb ischaemia. The effects of surgical resolution of limb ischaemia on thrombotic and rheological parameters in such persons has not previously been reported.

Aims:

1) To determine the effects of revascularisation or amputation surgery on the abnormal blood rheology observed in patients with critical limb ischaemia.

2) To determine whether or not levels of the potential thrombotic mediators plasma fibrinogen, fibrin degradation products (FDP's), von Willebrand factor (vWF), factor VII, plasminogen activator inhibitor (P.A.I.), and tissue plasminogen activator (t.P.A.), are altered by such surgical treatment of critical limb ischaemia.

3) To determine, by means of comparison with an age-matched random population sample, whether or not surgical resolution of critical limb ischaemia

results in the normalisation of previously abnormal blood rheology and levels of potential thrombotic mediators.

4) To determine whether or not the alterations in levels of thrombotic mediators following revascularisation in patients with critical limb ischaemia, are affected by the material used in the revascularisation procedure.

MATERIALS AND METHODS

Patients & methods

All the patients studied were in-patients at the vascular surgery units of Gartnavel General Hospital, and Glasgow Royal Infirmary. The diagnosis of critical limb ischaemia was made in accordance with the recommendations of the European Working Group on Critical Limb Ischaemia (1990).

Patients were invited to attend for review 12-16 weeks after surgical treatment, and resolution of critical limb ischaemia was confirmed by relief of rest pain, resolution of infection and tissue healing, and an increase in the ankle systolic pressure to above 50mm Hg. In patients who had undergone amputation, stump healing and relief of rest pain were taken as confirmatory of resolution of critical ischaemia.

Ankle systolic pressure was measured as described in chapter 2, and venous blood was sampled with minimal venous stasis in all patients prior to surgery for critical limb ischaemia, and again at review 12-16 weeks post-operatively. All samples were handled and assayed as described in chapter 2.

Controls

Control values for blood viscosity, red cell aggregation, and thrombotic mediators were obtained from local population studies (1st, 2nd, and 3rd W.H.O. Monica studies), as described in chapter 2, and the results for 80 patients aged between 65 and 75 years (mean age 69yrs.), were used as control values.

Statistics

All statistical analyses were performed by myself on a microcomputer, using the CSS: Statistica package (Statsoft, Tulsa, USA). Pre and post-operative results were compared using non-parametric Wilcoxon matched pairs testing, with pairwise deletion where individual data values were missing, and post-operative results were compared with population control values by means of the Mann-

Whitney U-test. Where comparisons were made on the basis of the graft material used patient characteristics were compared using the Chi-square test to identify any significant differences between the 2 patient groups.

RESULTS

Patients and outcomes

There were 82 patients enrolled in the study (51 male, 31 female), with a mean age of 70 years (range 50 - 96 years). 61 patients survived to review at 12 - 16 weeks post-operatively, giving an overall 16-week mortality rate of 26%. 5 of the 61 survivors were unable to attend for review (3 due to distances involved, 2 refused to attend), but were confirmed to be alive by their General Practitioners.

There was no evidence of recurrence of critical limb ischaemia in the 56 patients who attended for review, the majority having undergone a reconstructive procedure (Table 4.1, p.110). The mean age of these 56 patients was 69 years, with a range of 50 - 89 years, 29 patients had undergone insertion of a synthetic graft (PTFE or dacron), while in 23 patients autogenous vein had been used. On the basis of clinical history and estimation of plasma carboxyhaemoglobin levels, 38 of the 56 patients reviewed were current or recent smokers, and 18 had been non-smokers for more than 5 years. There were no significant differences in smoking habits between patients in whom vein grafts were used, and those in whom synthetic grafts were used ($\Sigma = 0.77$, Chi-Square test, 1 degree freedom), and in addition there were no significant differences in other patient details between the 2 patient groups (Table 4.2, p.111).

A number of patients were on warfarin therapy, and the Factor VII estimations in all those patients on warfarin therapy have been excluded from all analyses.

Blood rheology

Following resolution of critical limb ischaemia there was a significant fall in the relative blood viscosity ($p = 0.006$, Wilcoxon matched pairs), and a trend towards a significant fall in the corrected blood viscosity ($p = 0.07$), in the 56 patients available for review, together with significant increases in serum albumin and globulin (Table 4.3, p.112). Comparison of post-operative blood rheology in patients with age-matched population controls (Table 4.4, p.133), failed to reveal any significant differences, with the exception of the white cell count, which

OPERATION	NO. OF PATIENTS
Femoropopliteal/ femoro-distal graft	45
Limb amputation	4
Axillo-bifemoral graft	3
Patch profundaplasty	2
Femoro-femoral crossover graft	2

Table 4.1: Operations carried out in 56 patients with critical limb ischaemia (excludes 21 patients dying within 3 months of surgery, and 5 patients lost to follow up).

	VEIN GRAFT	SYNTHETIC GRAFT	AMPUTATION	TOTAL
	23	**29**	**4**	**56**
mean age (yrs)	69	69	68	69
prior vascular surgery	16	20	3	39
active sepsis/ tissue necrosis	11	11	3	25
diabetes	6	5	2	13
current/recent smoker	14	21	3	38
ex/non-smoker	9	8	1	18
Antiplatelet therapy				
pre-operative	5	9	0	14
post-operatively	5	11	0	16
Anticoagulant therapy				
pre-operatively	2	2	0	4
post-operatively	2	3	0	5

Table 4.2: Patient details in 56 patients with critical limb ischaemia, excluding 21 patients dying within 3 months of surgery, and 5 patients lost to follow up.

VARIABLE	PRE-OP. LEVEL	POST-OP. LEVEL	WILCOXON MATCHED PAIRS
White Cell Count ($x10^9/l$)	9.6 (8.0-12.2)	8.6 (7.0-10.2)	$p < 0.0001$
serum albumin (g/l)	38 (32-42)	41 (37-44)	$p = 0.0001$
serum globulin (g/l)	26 (23-29)	28 (26-31)	$p = 0.0007$
relative blood viscosity	2.50 (2.35-2.66)	2.37 (2.13-2.51)	$p = 0.006$
corrected blood viscosity (mPa.s)	3.43 (3.22-3.70)	3.27 (3.06-3.58)	$p = 0.07$
Red cell aggregation (units)	4.1 (3.0-5.5)	3.7 (3.0-4.4)	$p = 0.12$
haematocrit (%)	41 (37 - 45)	42 (38 - 45)	$p = 0.26$
plasma viscosity (mPa.s)	1.38 (1.31-1.50)	1.39 (1.33-1.48)	$p = 0.72$

Table 4.3: Changes in blood rheology following successful surgical treatment for critical limb ischaemia in 56 patients. Figures are median values (interquartile range).

VARIABLE	POPULATION LEVEL	POST-OP. LEVEL	MANN-WHITNEY U-TEST
White Cell Count ($x10^9/l$)	6.2 (5.1-7.9)	8.6 (7.0-8.2)	$p < 0.0001$
fibrinogen (g/l)	3.1 (2.7-3.8)	3.8 (3.0-4.9)	$p = 0.0006$
fibrin degradation products (ng/ml)	220 (115-310)	316 (192-620)	$p = 0.001$
von Willebrand factor (iu/dl)	120 (90-147)	153 (108-197)	$p = 0.002$
plasminogen activator inhibitor (% pool)	78 (60-92)	90 (69-121)	$p = 0.02$
tissue plasminogen activator (ng/ml)	8.4 (5.4-10.7)	6.9 (5.5-9.0)	$p = 0.06$
Red cell aggregation (units)	4.4 (3.5-5.0)	3.7 (3.0-4.4)	$p = 0.06$
plasma viscosity (mPa.s)	1.36 (1.30-1.44)	1.39 (1.33-1.48)	$p = 0.07$
factor VII (% pool)	113 (93-135)	110 (91-119)	$p = 0.12$
relative blood viscosity	2.42 (2.24-2.61)	2.37 (2.13-2.51)	$p = 0.13$
haematocrit (%)	44 (41 - 47)	42 (38 - 45)	$p = 0.26$
corrected blood viscosity (mPa.s)	3.40 (3.12-3.60)	3.27 (3.06-3.58)	$p = 0.46$

Table 4.4: Blood viscosity, red cell aggregation, and levels of thrombotic mediators following successful surgical treatment for critical limb ischaemia (56 patients), Compared with values in an age-matched population. Figures are median values (interquartile range). Factor VII levels in patients receiving warfarin have been excluded.

remained elevated in patients following revascularisation surgery. Relative blood viscosity and red cell aggregation were both lower following surgery, than in an age-matched population control, but the differences failed to attain statistical significance.

There were no other significant changes in blood rheology following correction of critical limb ischaemia, although in patients who had undergone insertion of an autogenous vein graft there were significant falls in the post-operative corrected blood viscosity ($p = 0.009$), and relative blood viscosity ($p = 0.005$) (Table 4.5, p.115) that were not observed in patients undergoing revascularisation with a synthetic graft (Table 4.6, Figures 4.1, 4.2, p.116-118), with post-operative relative blood viscosity (Figure 4.3, p.119) being significantly lower than in the population controls ($p = 0.03$).

Increases in albumin (Figure 4.4, p.120) and globulin (Figure 4.5, p.121) levels were seen in both vein and synthetic groups, as was the post-operative reduction in white cell count (Figure 4.6, p.122). Revascularisation procedures involving the use of a synthetic graft however, failed to alter rheological parameters post-operatively, with the exception of red cell aggregation, which fell significantly ($p = 0.006$) (Figure 4.3), to a level well below that seen in the control population ($p = 0.02$).

The presence of pre-operative tissue sepsis or necrosis may affect rheological parameters pre-operatively. Following successful resolution of critical limb ischaemia, these features are no longer present. However exclusion of such cases did not significantly influence the results, and significant falls in relative blood viscosity and red cell aggregation were still observed following resolution of critical limb ischaemia in this subgroup of patients (Table 4.7, p.123).

Thrombotic mediators

Levels of the potential thrombotic mediators plasma fibrinogen, von Willebrand Factor, and tissue plasminogen activator, all fell significantly following resolution of critical limb ischaemia (Table 4.8, p.124). Factor VII levels rose significantly following surgery ($p = 0.008$), while there was no significant change in the levels of cross-linked FDP's following surgery. Levels of most potential thrombotic mediators remained elevated in comparison with the control population, with the exception of factor VII and t.P.A. (Table 4.4, p.113).

Exclusion of patients with pre-operative sepsis or tissue necrosis from the analysis did not alter these observations (Table 4.9, p.125), with the exception of

VARIABLE	PRE-OP. LEVEL	POST-OP. LEVEL	WILCOXON MATCHED PAIRS
von Willebrand factor (iu/dl)	182 (124-237)	135 (108-197)	p= 0.001
relative blood viscosity	2.44 (2.36-2.52)	2.27 (2.13-2.42)	p= 0.005
corrected blood viscosity (mPa.s)	3.41 (3.21-3.60)	3.13 (2.99-3.41)	p= 0.009
fibrinogen (g/l)	4.7 (4.0-5.5)	3.7 (3.0-5.0)	p= 0.01
serum albumin (g/l)	37 (32-41)	41 (37-43)	p= 0.03
White Cell Count ($x10^9$/l)	8.5 (7.8-11.3)	8.2 (6.5-9.7)	p= 0.03
fibrin degradation products (ng/ml)	296 (215-575)	219 (141-534)	p= 0.09
serum globulin (g/l)	25 (20-29)	28 (24-29)	p= 0.14
factor VII (% pool)	109 (79-119)	110 (105-118)	p= 0.21
tissue plasminogen activator (ng/ml)	7.8 (5.3-13.0)	6.5 (5.5-7.7)	p= 0.35
haematocrit (%)	41 (37 - 45)	42 (39 - 46)	p= 0.42
Red cell aggregation (units)	3.9 (2.9-5.0)	3.9 (3.3-4.9)	p= 0.64
Platelet count ($x10^9$/l)	287 (235-350)	297 (239-331)	p= 0.68
plasminogen activator inhibitor (% pool)	103 (82-128)	99 (78-119)	p= 0.88
plasma viscosity (mPa.s)	1.36 (1.30-1.44)	1.38 (1.31-1.46)	p= 0.98

Table 4.5: Changes in blood rheology and potential thrombotic mediators following successful surgical treatment for critical limb ischaemia in 23 patients undergoing infra-inguinal vein grafts. Figures are median values (interquartile range).

VARIABLE	PRE-OP. LEVEL	POST-OP. LEVEL	WILCOXON MATCHED PAIRS
White Cell Count ($x10^9/l$)	10.9 (8.3-13.9)	9.1 (7.5-11.7)	p= 0.001
serum albumin (g/l)	39 (32-43)	41 (37-44)	p= 0.002
serum globulin (g/l)	26 (24-29)	29 (26-31)	p= 0.003
Red cell aggregation (units)	4.6 (3.8-6.2)	3.7 (3.0-4.3)	p= 0.006
factor VII (% pool)	100 (73-115)	114 (92-119)	p= 0.01
tissue plasminogen activator (ng/ml)	8.5 (7.0-10.0)	7.2 (5.3-9.8)	p= 0.01
fibrinogen (g/l)	4.6 (3.4-5.3)	3.7 (3.1-4.8)	p= 0.02
plasminogen activator inhibitor (% pool)	103 (77-133)	85 (60-121)	p= 0.06
von Willebrand factor (iu/dl)	177 (153-203)	164 (109-198)	p= 0.07
fibrin degradation products (ng/ml)	255 (196-463)	401 (252-724)	p= 0.09
relative blood viscosity	2.54 (2.30-2.83)	2.46 (2.25-2.65)	p= 0.42
Platelet count ($x10^9/l$)	328 (279-379)	325 (248-389)	p= 0.63
corrected blood viscosity (mPa.s)	3.43 (3.11-3.90)	3.37 (3.09-3.60)	p= 0.63
plasma viscosity (mPa.s)	1.37 (1.31-1.48)	1.40 (1.35-1.48)	p= 0.66
haematocrit (%)	40 (37 - 45)	40 (38 - 45)	p= 0.88

Table 4.6: Changes in blood rheology and potential thrombotic mediators following successful surgical treatment for critical limb ischaemia in 29 patients undergoing revascularisation surgery with a synthetic graft. Figures are median values (interquartile range).

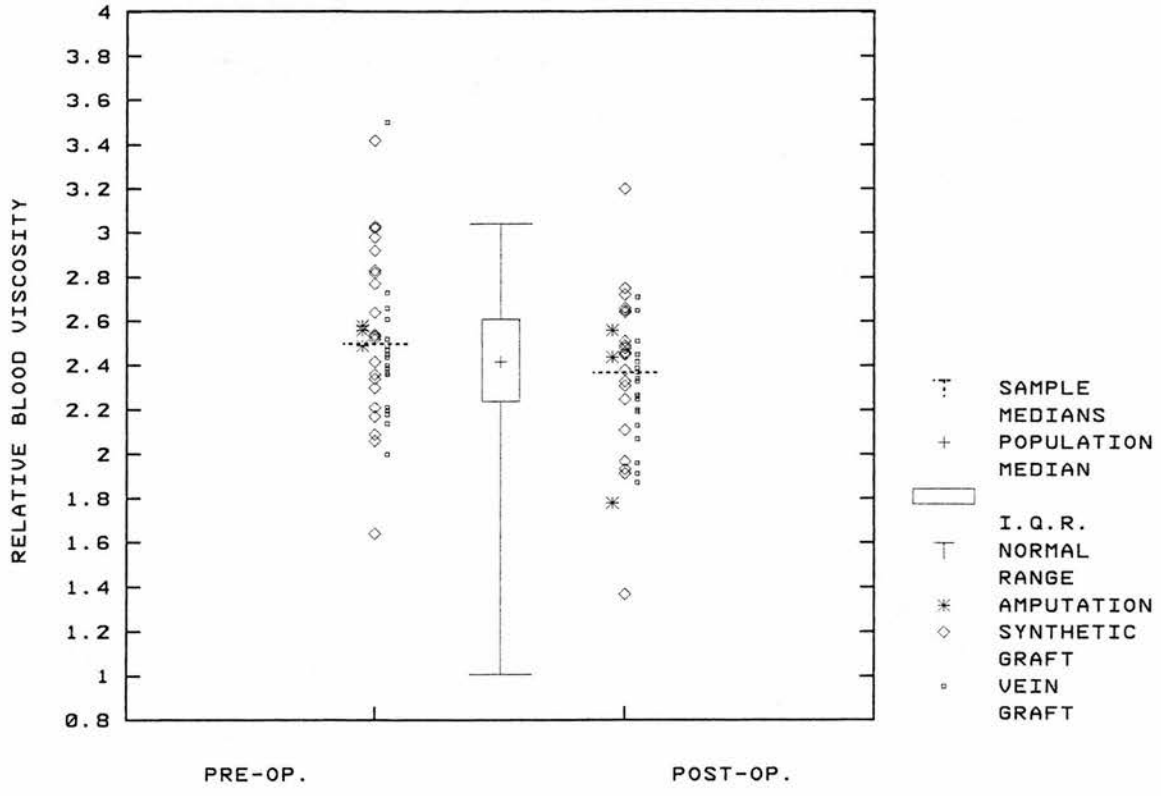


Figure 4.1: Changes in relative blood viscosity following correction of critical limb ischaemia.

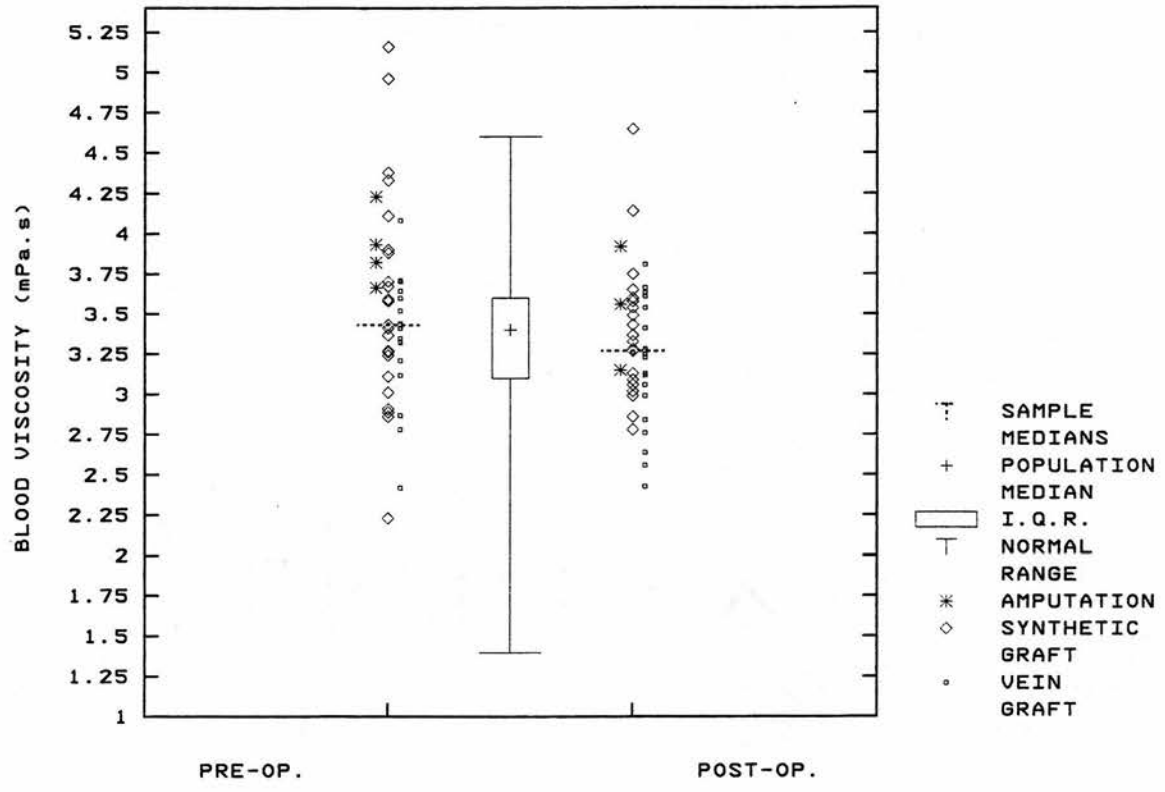


Figure 4.2: Changes in haematocrit-corrected blood viscosity following correction of critical limb ischaemia.

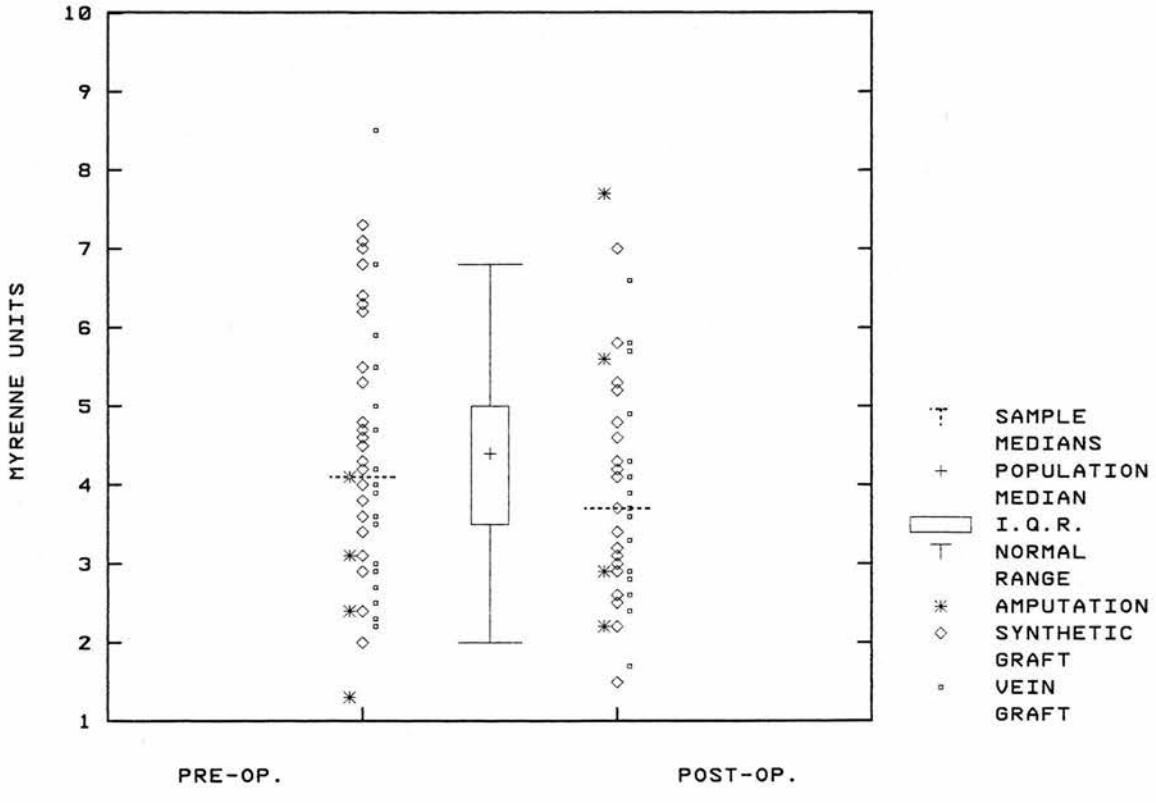


Figure 4.3: Changes in red cell aggregation following correction of critical limb ischaemia.

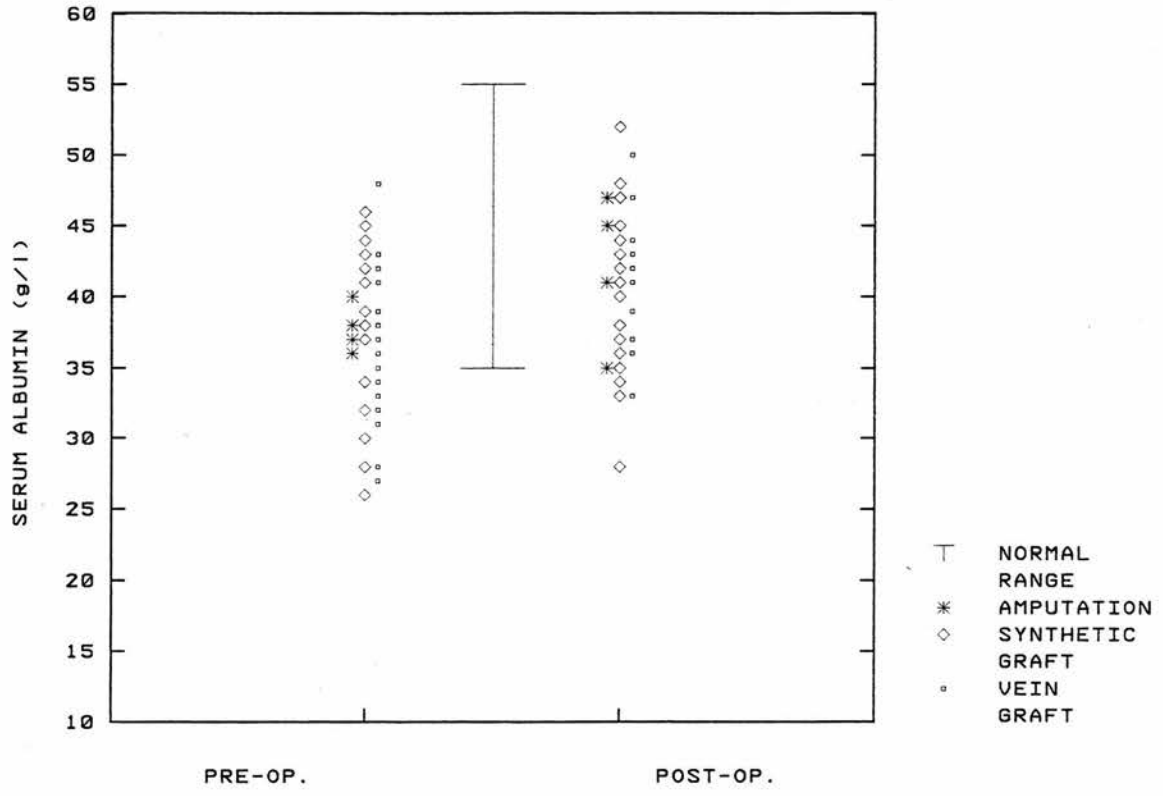


Figure 4.4: Changes in serum albumin levels following correction of critical limb ischaemia.

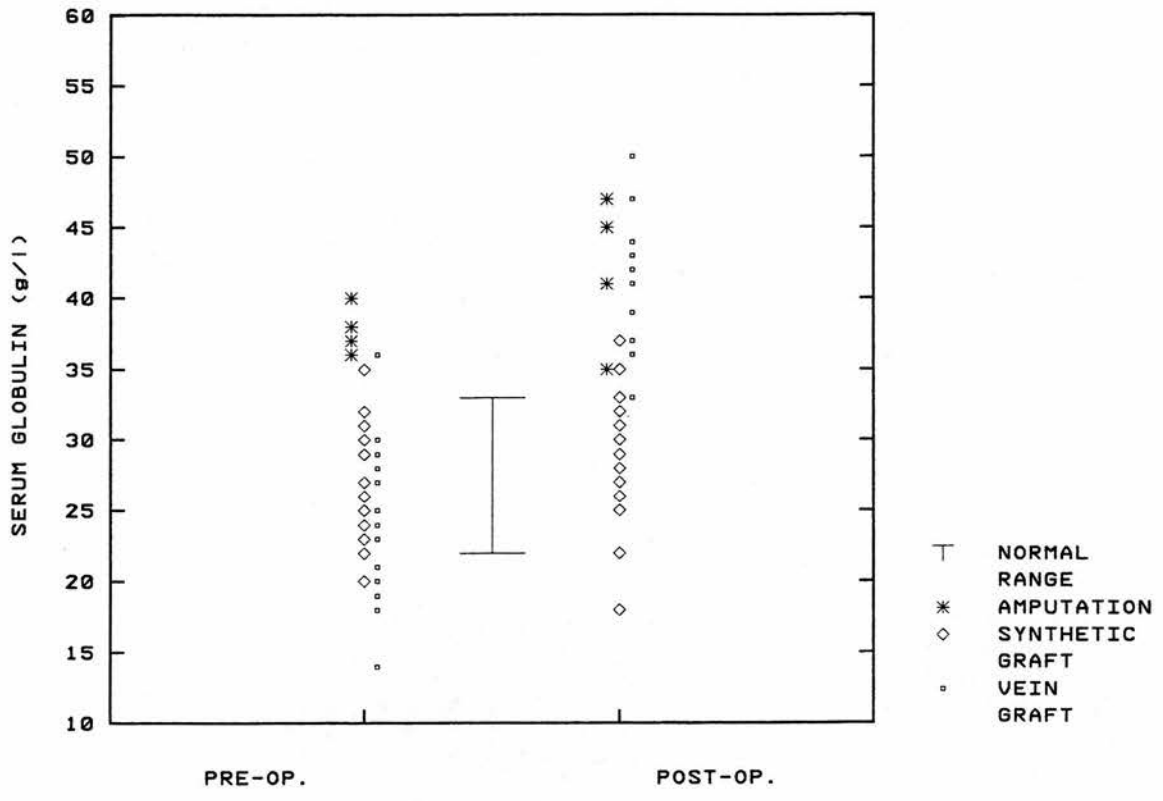


Figure 4.5: Changes in serum globulin levels following correction of critical limb ischaemia.

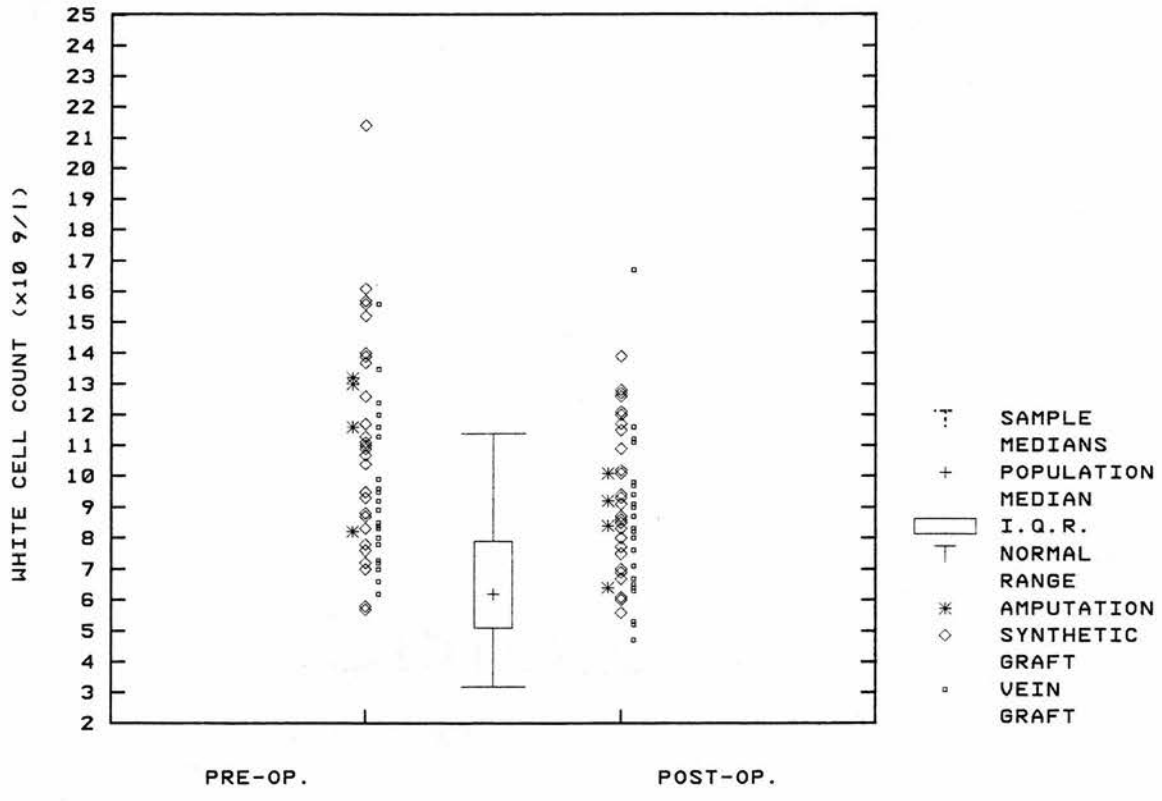


Figure 4.6: Changes in white cell count following correction of critical limb ischaemia.

VARIABLE	PRE-OP. LEVEL	POST-OP. LEVEL	WILCOXON MATCHED PAIRS
serum albumin (g/l)	38 (32-42)	43 (40-44)	$p < 0.002$
serum globulin (g/l)	25 (23-27)	28 (26-30)	$p < 0.005$
White Cell Count ($x10^9/l$)	9.3 (7.6-11.7)	8.7 (7.0-10.2)	$p = 0.009$
relative blood viscosity	2.49 (2.34-2.77)	2.34 (1.97-2.48)	$p = 0.02$
Red cell aggregation (units)	4.2 (3.6-5.5)	3.9 (3.2-4.8)	$p = 0.03$
haematocrit (%)	40 (37 - 45)	42 (37 - 46)	$p = 0.17$
corrected blood viscosity (mPa.s)	3.41 (3.12-3.67)	3.19 (2.99-3.58)	$p = 0.25$
plasma viscosity (mPa.s)	1.36 (1.30-1.41)	1.38 (1.35-1.43)	$p = 0.45$

Table 4.7: Changes in blood rheology following successful surgical treatment for critical limb ischaemia in 31 patients without pre-operative infection or tissue necrosis. Figures are median values (interquartile range).

VARIABLE	PRE-OP. LEVEL	POST-OP. LEVEL	WILCOXON MATCHED PAIRS
factor VII (% pool)	106 (79-116)	113 (98-119)	p= 0.004
fibrinogen (g/l)	4.7 (3.6-5.5)	3.8 (3.0-4.9)	p< 0.005
von Willebrand factor (iu/dl)	177 (144-226)	153 (108-197)	p< 0.005
tissue plasminogen activator (ng/ml)	8.5 (6.5-11.8)	6.9 (5.5-9.0)	p= 0.006
plasminogen activator inhibitor (% pool)	103 (77-130)	90 (69-121)	p= 0.12
Platelet count ($x10^9$/l)	314 (243-368)	303 (244-351)	p= 0.38
fibrin degradation products (ng/ml)	285 (225-510)	316 (192-620)	p= 0.76

Table 4.8: Changes in levels of platelets and potential thrombotic mediators following successful surgical treatment for critical limb ischaemia in 56 patients. Figures are median values (interquartile range). Factor VII levels in patients receiving warfarin have been excluded.

VARIABLE	PRE-OP. LEVEL	POST-OP. LEVEL	WILCOXON MATCHED PAIRS
fibrinogen (g/l)	4.2 (3.4-5.3)	3.3 (3.0-4.4)	p= 0.001
von Willebrand factor (iu/dl)	175 (118-223)	134 (97-200)	p= 0.002
factor VII (% pool)	104 (80-116)	114 (104-122)	p= 0.01
tissue plasminogen activator (ng/ml)	8.8 (6.9-12.0)	7.0 (5.5-9.8)	p= 0.07
fibrin degradation products (ng/ml)	260 (214-519)	409 (202-768)	p= 0.16
plasminogen activator inhibitor (% pool)	90 (76-122)	90 (72-125)	p= 0.44
Platelet count ($x10^9$/l)	289 (232-334)	287 (239-338)	p= 0.80

Table 4.9: Changes in thrombotic mediators following successful surgical treatment for critical limb ischaemia in 31 patients without pre-operative infection or tissue necrosis. Figures are median values (interquartile range).

the fall in t.P.A. levels on resolution of critical limb ischaemia, which failed to reach statistical significance after exclusion of sepsis and gangrene ($p = 0.07$).

Changes in the levels of thrombotic mediators varied with the material employed for revascularisation: Although both plasma fibrinogen (Fig. 4.7, p.127) and vWF (Fig. 4.8, p.128) levels fell following revascularisation with both vein and synthetic grafts, there were no significant changes in the levels of factor VII, t.P.A., and P.A.I. following insertion of a vein graft (Table 4.5, p.115). There was however a trend towards a significant fall in the post-operative levels of cross-linked FDP following insertion of a vein graft ($p = 0.09$), in contrast to the trend towards a significant rise observed after insertion of a synthetic graft ($p = 0.09$) (Figure 4.9, p.129).

Insertion of a synthetic graft was also associated with significant falls in plasma fibrinogen, t.P.A. (Fig. 4.10, p.130) and P.A.I. (Fig. 4.11, p.131) levels, a trend towards a significant fall in vWF levels ($p = 0.07$), and a rise in factor VII levels (Fig. 4.12, p.132), following resolution of critical limb ischaemia (Table 4.6, p.116). Following revascularisation with a synthetic graft post-operative levels of cross-linked FDP's were significantly higher than those seen in the population controls ($p < 0.01$).

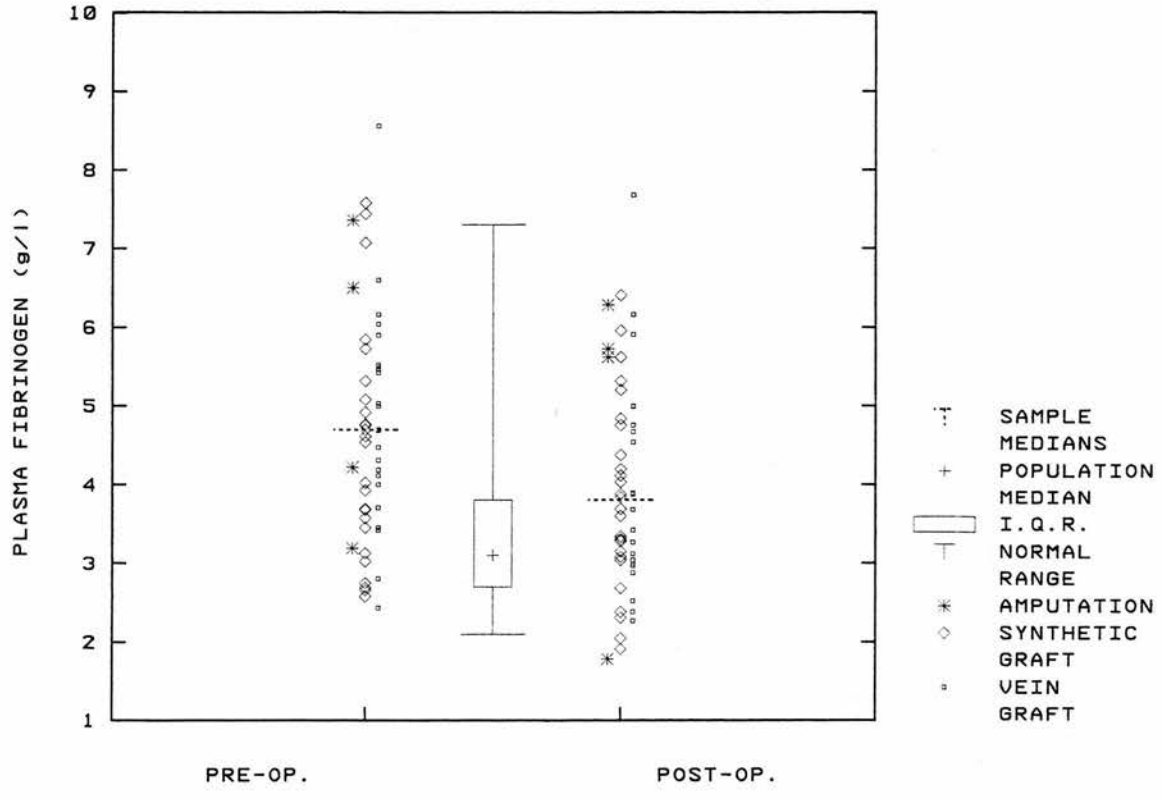


Figure 4.7: Changes in plasma fibrinogen levels following correction of critical limb ischaemia.

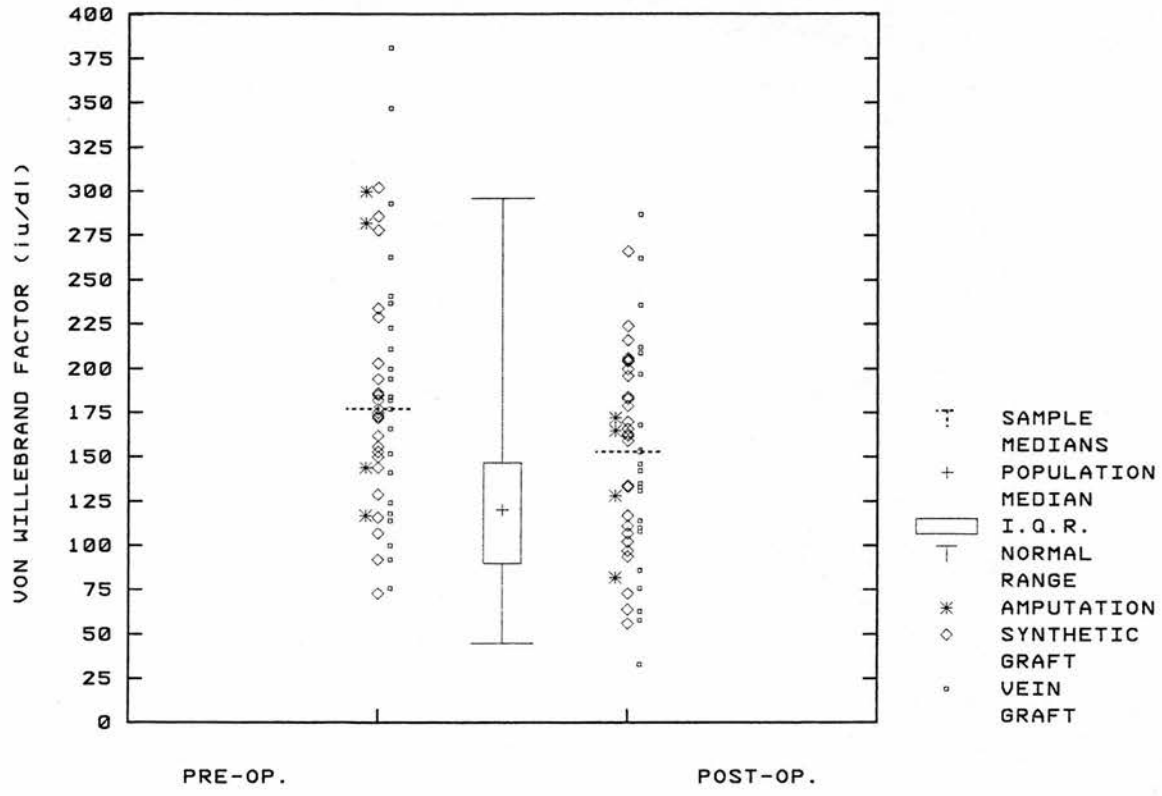


Figure 4.8: Changes in plasm von Willebrand Factor following correction of critical limb ischaemia.

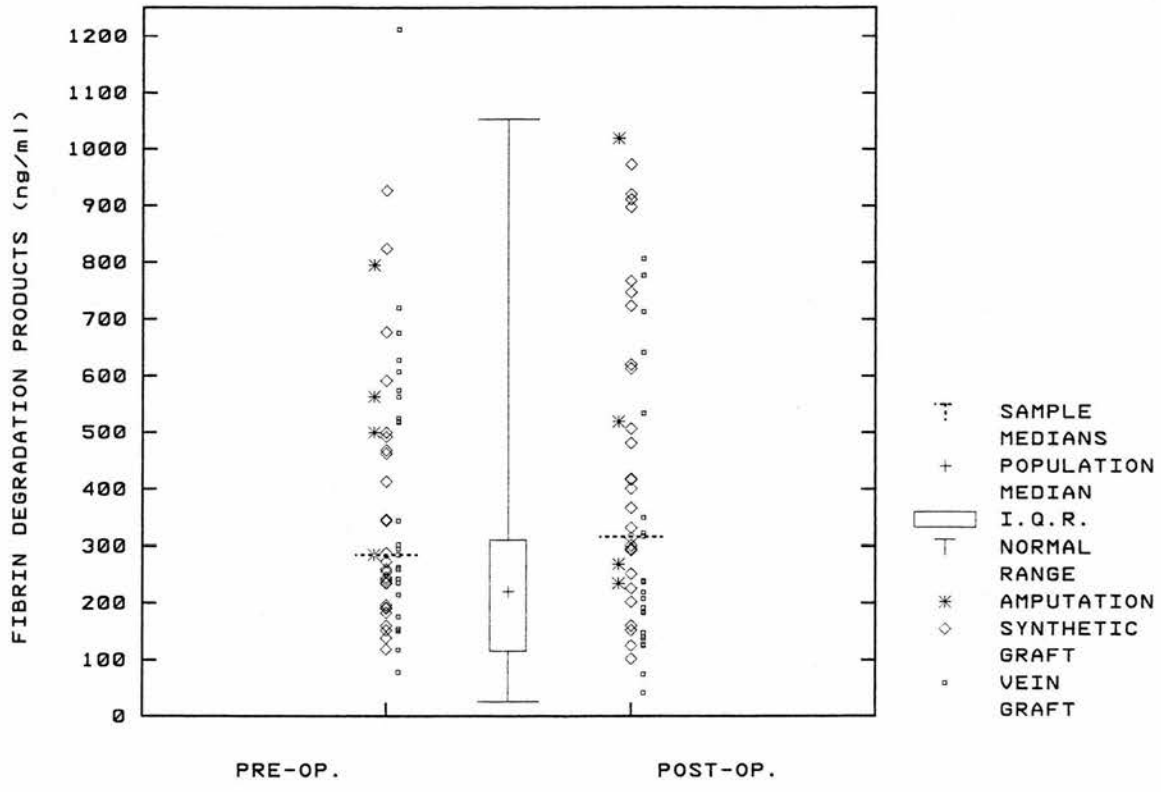


Figure 4.9: Changes in cross-linked fibrin degradation products following correction of critical limb ischaemia.

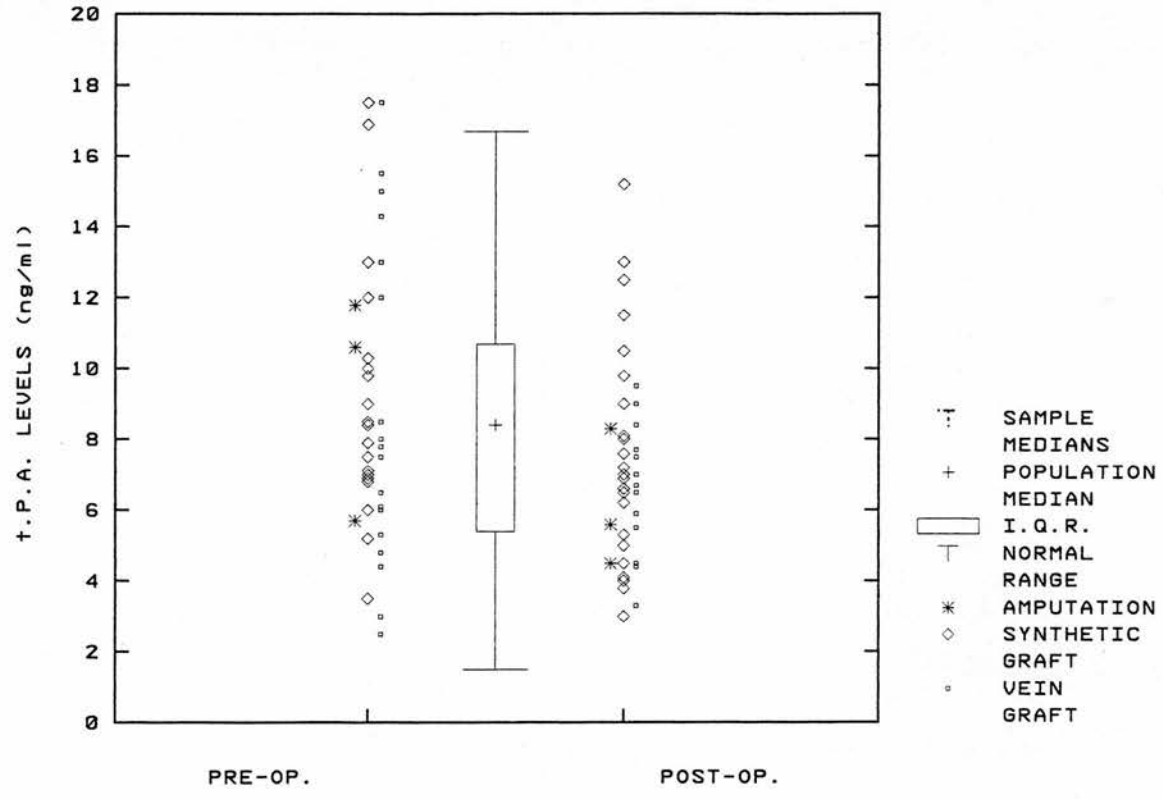


Figure 4.10: Changes in tissue plasminogen activator (t.P.A.) following correction of critical limb ischaemia.

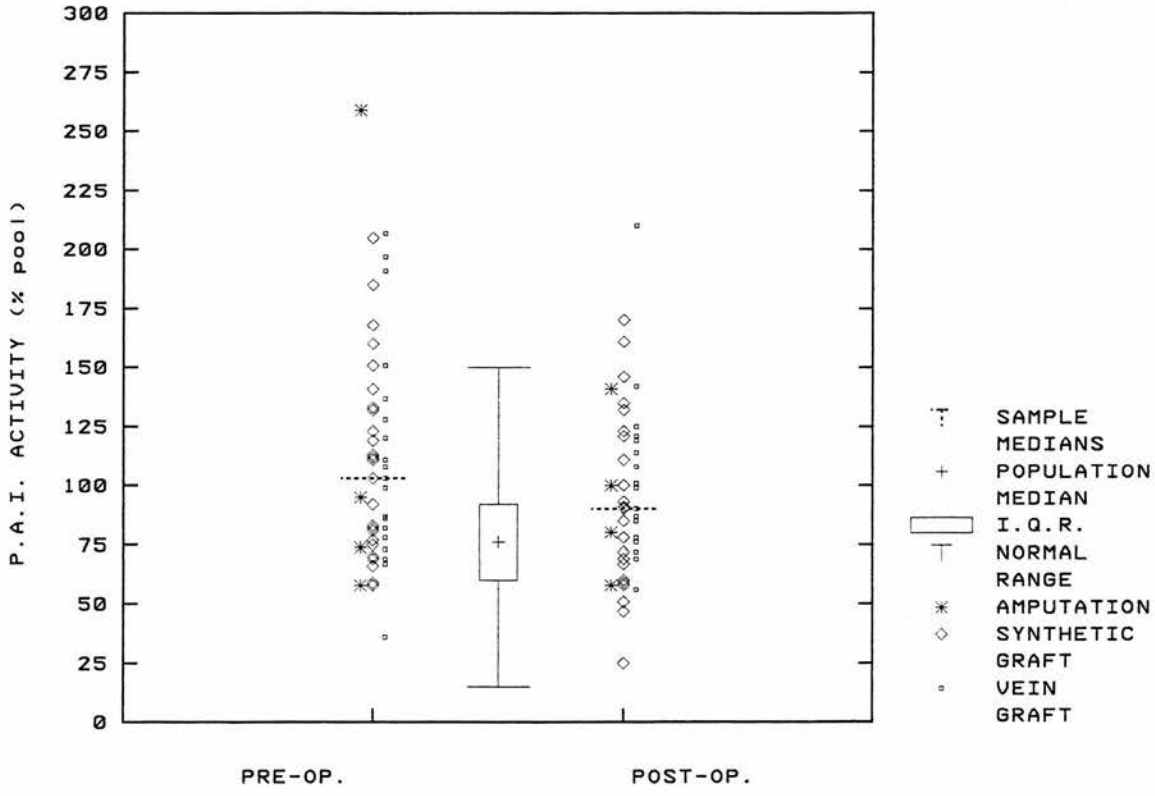


Figure 4.11: Changes in plasminogen activator inhibitor (P.A.I.) levels following correction of critical limb ischaemia.

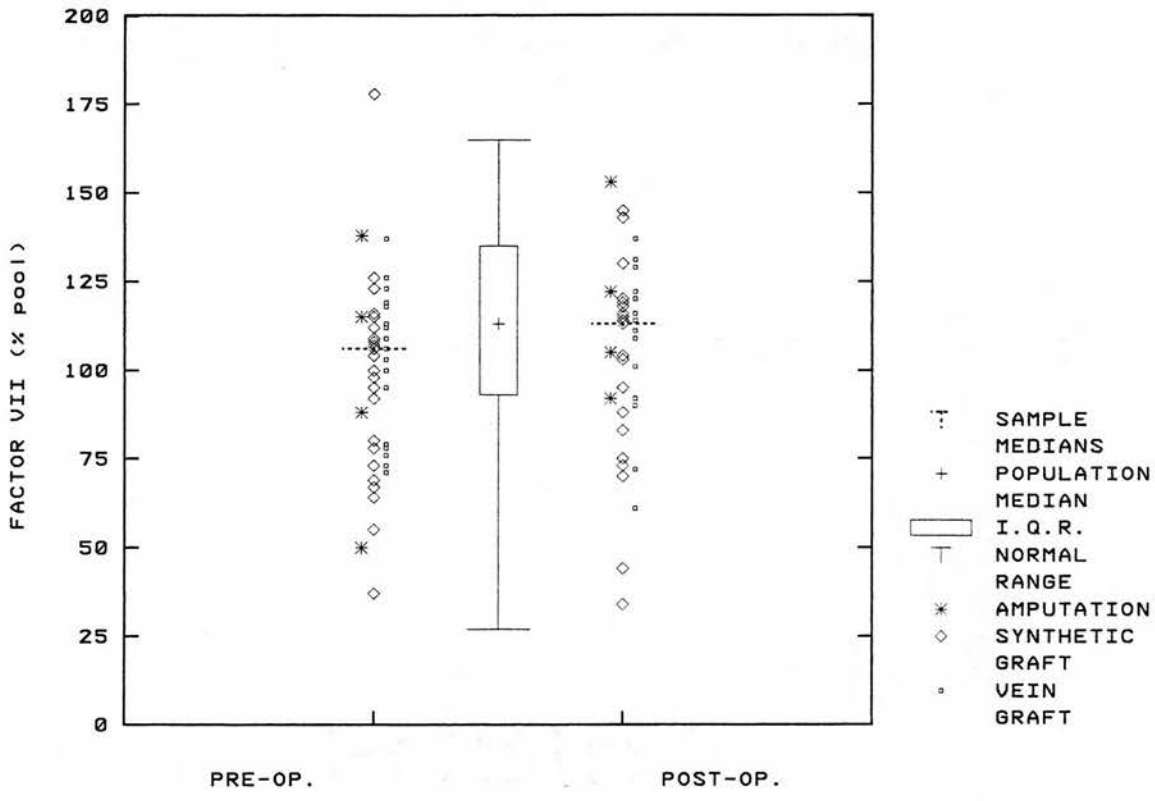


Figure 4.12: Changes in factor VII levels following correction of critical limb ischaemia.

DISCUSSION

Patients & revascularisation procedure

Although this group of patients were not randomly selected, and are heavily biased towards cases of infra-inguinal disease, they are typical of many patients with advanced symptomatic occlusive arterial disease, the majority of whom have infra-inguinal disease (Bloor, 1961, Hertzer et al, 1991), either in isolation or as a component of multi-level disease (Bell, 1990). An early mortality rate of 26% in this series is high, but there are few figures available for comparison; the mortality rate from amputation surgery ranges from 8 to 18% (Harris & Moody, 1990) depending on the site of amputation, and it is reported that at least 20% of patients with critical limb ischaemia die within 1 year of diagnosis (Norgren, 1990). Reliable data for comparison is however limited, and this study is more concerned with the effects of successful resolution of critical limb ischaemia on blood rheology and potential thrombotic mediators.

The observation that 39 of the 56 survivors had undergone prior revascularisation procedures is in keeping with the finding in chapter 3 that a history of prior vascular surgery is independently associated with the angiographic severity of peripheral arterial disease. One would expect patients developing critical limb ischaemia as a consequence of chronic occlusive arterial disease to have undergone prior revascularisation surgery for symptoms of claudication and critical limb ischaemia, while the significant number of patients undergoing *de novo* vascular reconstruction for critical limb ischaemia in this series is due to both social factors leading to late presentation, and the individual surgeons decision to defer surgery in some patients until the development of limb threat.

The 23% incidence of diabetes in the patients studied is approximately 5 times the incidence of diabetes in the general population (Krone & Müller-Wieland, 1990), and reflects the high incidence of gangrene in these patients (Gutman et al, 1987), while the observation that over two thirds of the patients were current or recent smokers confirms the strong association between smoking and arterial disease (Hughson, 1978, Fowkes, 1989).

The use of aspirin as antiplatelet therapy either prior to, or following surgery, in a number of patients is unlikely to influence the results of this study as previous work has demonstrated that aspirin therapy has no effect on blood rheology or thrombotic mediators (Reid, 1991), while the exclusion of factor VII results from patients on warfarin therapy ensures that pre- and post-operative

comparisons are accurate. The effect of warfarin on blood rheology and other thrombotic mediators is not significant (Reid, 1991).

Blood rheology

In this group of patients there were surprisingly few changes in rheological parameters following resolution of critical limb ischaemia, with only red cell aggregation and relative blood viscosity (blood viscosity corrected for the effects of haematocrit and plasma viscosity) showing a significant fall post-operatively. Comparison of post-operative rheological values with age-matched population controls indicates that values return to normal following resolution of critical limb ischaemia.

There is a trend towards a significant elevation in plasma viscosity following correction of critical limb ischaemia, despite a fall in plasma fibrinogen, the major determinant of plasma viscosity (Lowe, 1991). This may be a consequence of increased levels of albumin and serum globulin, which rose significantly on resolution of critical limb ischaemia, thus offsetting the effect of the fall in plasma fibrinogen.

Reduced haematocrit and plasma protein levels in patients with critical limb ischaemia are in contrast to findings in claudicants (Lowe et al, 1986), and suggest that patients with critical limb ischaemia have a haematological picture more typical of patients with a chronic disease state (Reizenstein, 1979), with attendant reductions in serum protein levels. This reduction in serum protein levels may result in the lowering of plasma viscosity, which has previously been shown to be elevated in patients with claudication (Lowe et al, 1986, 1993). Thus occlusive arterial disease leads to an increase in blood and plasma viscosity (Lowe et al, 1993) with increasing severity of disease, until a state of chronic arterial insufficiency results in critical limb ischaemia. At some point along this continuum, the elevations in blood and plasma viscosity are offset by the haematological response to a chronic disease state which lowers plasma proteins and haematocrit.

The only significant rheological response to the correction of critical limb ischaemia observed in this study was a fall in relative blood viscosity, although closer analysis indicated that this fall was only observed in cases where autogenous vein graft was used. In addition vein grafting was associated with a significant fall in haematocrit-corrected blood viscosity. This fall in relative blood viscosity (which is a measure of the cellular contribution to whole blood viscosity) reflects an

increase in red cell deformability following vein grafting, as has previously been observed after reconstructive vascular surgery (Irwin et al, 1983).

The significant fall in blood viscosity in association with the use of vein grafts is occurring in spite of the fact that post-operative levels of the plasma proteins albumin and globulin are increased, and that the increase in protein levels is most marked in vein grafts. This fall is therefore not related to alterations in the major plasma proteins, and in view of the similarity between fibrinogen levels following vein and synthetic grafting, is not related to fibrinogen either. It may be that differences in fibrin turnover, as reflected by FDP levels, are at least partly responsible for the fall in blood viscosity seen following insertion of a vein graft, and this is discussed in more detail below.

Plasma fibrinogen

The association between elevated plasma fibrinogen and atherosclerotic vascular disease has been reported by a number of authors (Dormandy et al, 1973a, Meade et al, 1986, Kannel et al, 1987, Ernst 1991), and there is evidence that the degree of elevation is related to the severity of the ischaemia (Lowe et al. 1991, Reid, 1991), as well as the observation that patients with peripheral arterial occlusive disease and elevated plasma fibrinogen show the most rapid disease progression (Dormandy et al. 1973B). This study suggests that relief of critical limb ischaemia reverses some of the elevation in plasma fibrinogen that is associated with advanced arterial occlusive disease, but that correction of critical limb ischaemia fails to reduce fibrinogen to the levels encountered in an age-matched population.

The inability to reduce plasma fibrinogen levels to normal after relief of critical limb ischaemia may be due to a number of factors: Plasma fibrinogen levels are elevated in smokers (Meade et al, 1979), and the majority of the patients studied here were smokers whose smoking habit remaining unchanged following surgery. Patients with advanced PAOD have widespread arterial involvement and correction of critical ischaemia in one limb does not eradicate their arterial disease: it therefore follows that these patients still have elevations in plasma fibrinogen as a consequence of occlusive arterial disease at other sites. An additional factor in the persistent elevation of plasma fibrinogen following surgery, may be related to genetic influences on plasma fibrinogen (Hamsten et al, 1987A, Fowkes et al, 1992, Meade, 1992) which may predispose certain individuals to elevated fibrinogen levels regardless of the state of their arterial circulation.

The type of graft material used did not seem to affect fibrinogen levels following resolution of critical ischaemia, suggesting that after an initial acute inflammatory response to the insertion of synthetic graft material (Reid, 1991), the presence of synthetic graft material no longer stimulates increased fibrinogen production. This may be related to formation of the layer of fibrin (and occasionally endothelial cells) that completely covers the graft surface in contact with blood (DeBakey et al, 1964).

The post-operative fall in plasma fibrinogen was not solely due to resolution of limb sepsis and the excision of necrotic and gangrenous tissue, although fibrinogen levels were lower in the non-septic, non-necrotic group of patients, confirming that the presence of sepsis and tissue necrosis leads to elevations in plasma fibrinogen (Meade, 1992).

These findings indicate that some of the elevation in plasma fibrinogen associated with critical limb ischaemia can be reversed by revascularisation (or amputation) of the affected limb, implying that biochemical changes stimulating fibrinogen synthesis occur in critically ischaemic tissues. This fibrinogen synthesis is probably hepatic in origin, in response to stimulation of hepatocytes by interleukin-6 (IL-6) (Cook & Ubben, 1990, Reid, 1991), produced by activated monocytes (Cruickshank et al, 1990) and it is likely that the fall in plasma fibrinogen following revascularisation is at least partly a result of the concurrent reduction in white cell activation that occurs on resolution of critical limb ischaemia, although further studies of white cell activation are required to clarify this point.

This fall in plasma fibrinogen following resolution of critical limb ischaemia has a number of potential clinical benefits: elevated plasma fibrinogen is known to be an independent risk factor for stroke and myocardial infarction (Ernst, 1991), as well as cardiovascular mortality (Meade et al, 1986), and also to be associated with arterial graft occlusion (Wiseman et al, 1989), and the reduction of plasma fibrinogen levels associated with correction of critical limb ischaemia may reduce the risk of these events occurring post-operatively.

Fibrin degradation products

Cross-linked fibrin degradation products (FDP's) are the result of lysis of cross-linked fibrin, and elevated levels of FDP's reflect an increase in fibrin turnover (Whittaker et al. 1987). Recent studies have suggested that FDP's are elevated in peripheral arterial disease (Reid, 1991), and that the elevation in FDP's is related to the severity of occlusive arterial disease (Peltonen et al, 1992,

Woodburn et al, 1993A). The finding that FDP's are independently associated with the severity of arterial disease, and the possible relevance of this to the aetiology of peripheral arterial disease has already been discussed.

Initial analysis of the results obtained in this study indicates that fibrin turnover is unaffected by successful resolution of critical limb ischaemia, with levels of FDP's remaining higher than that of an age-matched population. However graft type has a major effect on FDP levels following resolution of critical limb ischaemia.

The use of autologous vein to revascularise a critically ischaemic limb produces a trend towards a significant fall in FDP's, with post-operative levels comparable to those in the control population, indicating that increased fibrin turnover is a reversible feature of critical limb ischaemia when the limb is revascularised with an autogenous vein graft. This reduction in fibrin turnover may result from a reduction in thrombus formation secondary to stasis (Anderson, 1985), consequent on an improved arterial inflow. Any reduction in thrombus formation will reduce fibrin turnover, and therefore reduce levels of cross-linked FDP's.

In contrast, use of synthetic graft materials (PTFE and Dacron) to correct critical limb ischaemia was associated with a rise in cross-linked FDP levels that failed to attain statistical significance. A rise in FDP levels at one week following insertion of a synthetic graft has previously been reported (Reid, 1991), however the results in this study imply that fibrin turnover is greatly increased for at least 4 months after insertion of a synthetic graft, and this cannot therefore be attributed solely to surgical trauma, nor can it be related to increases in plasma fibrinogen levels, which fall significantly over the same time period in the same patients.

This increase in fibrin turnover probably occurs in all patients in whom a synthetic graft is inserted, regardless of the indication for surgery, and this is explored in more detail in Chapter 6. Previous studies have shown that the innermost layer coating the luminal surface of a synthetic graft consists almost entirely of fibrin (DeBakey et al, 1964), and these new findings suggest that this must be a dynamic layer that is continually being lysed and reformed. It may be that this "dynamic equilibrium" between fibrin formation and lysis (Anderson, 1985) that results in increased FDP's in patients revascularised with a synthetic graft, the increased fibrin turnover preventing accumulation of fibrin within the graft lumen that could lead ultimately to occlusion.

von Willebrand Factor

Between 75 and 85% of circulating vWF is derived from endothelial cells, where it is synthesised and stored, the remaining plasma von Willebrand factor being derived from platelet activation (Ingerslev, 1990). Elevated levels of vWF are therefore largely indicative of endothelial damage, and have been shown to be elevated following direct arterial injury (Woodburn et al, 1993B). In addition population studies show elevated vWF in the presence of peripheral arterial disease (Christe et al, 1984, Blann & McCollum, 1992A, Smith et al, 1993). Although little is known about the regulation of vWF levels (Ingerslev, 1990), it has been reported that vWF is elevated in inflammatory conditions (Blann et al, 1992), although the changes in vWF levels following revascularisation that were observed in this study were still present when cases with pre-operative sepsis and tissue necrosis were excluded, suggesting that the changes observed are not just a consequence of the resolution of infection and its associated inflammatory response.

Levels of vWF after vein grafting remain higher than in population controls, and this may be consequent upon increased cigarette consumption in the patient group. Cigarette smoking is known to elevate vWF levels (Blann, 1992). The presence of widespread arterial disease elsewhere in the vascular tree, with its associated endothelial damage promoting increased plasma vWF levels, may also contribute to the elevated vWF levels in the patient group. The post-operative fall in vWF may be the result of exclusion from the vascular tree of the diseased vessels bypassed in the revascularisation surgery. This may eliminate the release of endothelial cell vWF from these segments into the plasma, and reduce platelet-endothelial contact and activation, with a reduction in vWF release (Ingerslev, 1990).

Median plasma levels of vWF fall to lower levels following vein grafting than after synthetic grafting, and this may be related to increased levels of platelet activation, and hence vWF release (Ingerslev, 1990), in the presence of a synthetic graft. The increase in fibrin turnover associated with insertion of a synthetic graft could also lead to release of vWF stored in endothelial cells (Ribes et al, 1987). These mechanisms may therefore explain why vWF does not fall as markedly after synthetic grafting to resolve critical limb ischaemia, as it does in situations where autologous vein has been used, although further work is required to clarify this.

In spite of this graft-related difference in vWF levels following resolution of critical limb ischaemia, it is apparent that resolution of critical limb ischaemia will significantly reduce the abnormal elevation in plasma vWF associated with severe

ischaemia. This reduction in vWF levels may have implications for the subsequent progression of atherosclerosis in patients with peripheral arterial disease, as a reduction in vWF levels may reduce platelet-vessel wall interactions, which appear to be central to the progression of arterial disease (Badimon & Fuster, 1992).

P.A.I. and t.P.A.

Fibrinolysis is controlled in part by the level of activity of plasmin, formed from its inactive precursor, plasminogen, by the action of tissue-type Plasminogen Activator (t.P.A.) (Loscalzo, 1992). Activity of t.P.A. is however inhibited by glycoprotein Plasminogen Activator Inhibitors (P.A.I.) (Kruithof, 1988), and plasma levels of P.A.I. and t.P.A. therefore exert a major influence on fibrin turnover.

Resolution of critical limb ischaemia leads to a significant fall in t.P.A. levels and a less marked fall in P.A.I. levels. P.A.I. however behaves like an acute-phase protein (Hamsten & Wiman, 1992), and exclusion of cases with pre-operative sepsis or necrosis, indicates that while t.P.A. levels still fall after resolution of critical limb ischaemia, there is no fall in P.A.I. levels. These findings suggest that conversion of plasminogen to plasmin is reduced following resolution of critical limb ischaemia, and this may be due to a reduction in arterial and venous stasis, and consequently thrombus formation, on increasing blood flow to the affected limb(s).

The fall in tissue-type Plasminogen Activator levels on resolution of critical limb ischaemia, is independent of the type of graft utilised, although it only attains statistical significance after synthetic grafting. In contrast, P.A.I. levels show little change after insertion of a vein graft, while they fall markedly after insertion of a synthetic graft. This may reflect an increase in plasmin formation (and therefore fibrin turnover) in association with the use of synthetic grafts to revascularise critically ischaemic limbs. This would concur with the increase in fibrin turnover and cross-linked FDP levels already observed in this particular group of patients.

The increased rate of fibrin turnover in synthetic grafts may cause the associated fall in t.P.A. and P.A.I. levels. An alteration in equilibrium between t.P.A. and P.A.I. may be more important than their absolute plasma levels, in terms of their influence on fibrinolysis. What these results do indicate is that resolution of critical limb ischaemia does not alter this equilibrium significantly if autologous vein is used, but that alterations occur in the presence of a synthetic graft. In all cases, resolution of critical limb ischaemia reduces plasma t.P.A. levels to those seen in an age-matched population.

Factor VII

Factor VII levels are affected by a number of non-specific stimuli that result in an inverse relationship between Factor VII and fibrinogen levels (Meade, 1991). It is therefore not surprising that the fall in fibrinogen after resolution of critical limb ischaemia is accompanied by a rise in Factor VII levels, that is seen even after exclusion of pre-operative sepsis and necrosis. It appears that surgical resolution of critical limb ischaemia results in the return of factor VII levels to normal population levels, regardless of the material used for revascularisation, although the magnitude of the rise is smaller after insertion of a vein graft.

There are unlikely to be any significant implications of these changes in Factor VII. Critical limb ischaemia results in certain inflammatory changes (Lowe, 1990A), that result in Factor VII levels falling below those of an age-matched population. Resolution of critical limb ischaemia reverses these changes, with Factor VII levels returning to normal, and it is unlikely that these changes have a major effect on the overall thrombotic tendency, which is affected to a greater extent by the marked changes in other potential thrombotic mediators occurring in association with surgical treatment of critical ischaemia.

Summary

The work reported in this study indicates that several abnormalities of blood rheology and potential thrombotic mediators found in association with critical limb ischaemia, can be reversed by resolution of the limb ischaemia. However only a minority of these variables are returned to a level that corresponds to that seen in an age-matched population.

1 in 4 of the patients undergoing treatment for critical limb ischaemia are dead within 4 months of surgery. This observation has implications for patient selection and requires further study.

Relative blood viscosity, an index of red cell deformability, is reduced following resolution of critical limb ischaemia, and this has been observed in a prior study (Irwin et al, 1983). This reduction is not seen in patients revascularised with a synthetic graft.

Blood and plasma viscosity are essentially unaltered after resolution of critical limb ischaemia despite a large fall in plasma fibrinogen, and it is postulated that the effect of this fall is offset by the increase in other plasma protein levels which follows surgery.

This fall in plasma fibrinogen implies that critical limb ischaemia leads to an increase in fibrinogen synthesis that is partly reversible. This could be related to hepatic stimulation by products of activated monocytes. The persisting elevation in fibrinogen following revascularisation may reflect a genetic predisposition to high fibrinogen in patients who develop critical limb ischaemia, or generalised atherosclerosis.

Cross-linked fibrin degradation product levels are unchanged following revascularisation with autologous vein, but are greatly increased following insertion of a synthetic graft, and this is likely to reflect the continuous turnover of the fibrinous layer lining these grafts.

von Willebrand factor levels fall with revascularisation, but fail to return to population levels, possibly because the widespread nature of peripheral arterial disease results in the continued contact between blood and damaged endothelium. Levels of vWF are higher after revascularisation with synthetic grafts and this may reflect increased platelet activation and degranulation in the presence of such grafts. Further studies of markers of platelet activation need to be carried out in such patients.

The regulators of fibrinolysis, t.P.A. and P.A.I., are reduced following revascularisation surgery, although insertion of a synthetic graft reduces P.A.I. to a greater extent, suggesting that the balance between activation and inhibition of

plasminogen is shifted in favour of activation. This observation would be in keeping with the postulated increase in fibrin turnover accompanying the insertion of a synthetic graft.

Factor VII levels rise after revascularisation and this is probably due to the tendency of Factor VII to behave as a "negative acute-phase reactant". This return to normal population levels is unlikely to have a significant effect on an individual's thrombotic tendency following revascularisation.

CHAPTER 5

The effect of percutaneous angioplasty on blood rheology and thrombotic mediators in peripheral arterial disease

INTRODUCTION

Percutaneous transluminal angioplasty (PTA) is now an established method of treating arterial stenoses in both peripheral and coronary arterial disease, and the results of PTA in peripheral arterial disease have been extensively reviewed (Dake & Katzen, 1990), although meaningful comparison between different series is hampered by the lack of uniform reporting methods (Rutherford & Becker, 1991). The lesions most suited to percutaneous angioplasty have also been identified (Dake & Katzen, 1990, Al-Kutoubi, 1992), the cost benefits of the procedure have been emphasised (Doubilet & Abrahams, 1984), and the clinical indications for percutaneous angioplasty have been at least partly defined (Campbell, 1986, Whyman et al, 1991).

Studies of the direct effects of percutaneous angioplasty have been limited to pathophysiological studies of the mechanism of angioplasty (Castenada-Zuniga et al, 1980, Zarins et al, 1982, Kinney et al, 1984) that suggest that the process is one of controlled injury produced by dilatation, followed by a healing process that results in a smooth-walled lumen (Dake & Katzen, 1990). The effects of angioplasty on blood rheology and thrombotic mediators, which are associated with peripheral arterial disease (Lowe, 1986, Lowe, 1992, Leng & Fowkes, 1991B, Woodburn et al, 1993A), has not been studied. This chapter examines the effects of percutaneous angioplasty on blood rheology and potential thrombotic mediators, and also compares arterial and venous levels of these variables.

Aims:

1) To determine whether or not measures of blood rheology, fibrin turnover (cross-linked FDP's), von Willebrand Factor (vWF), Plasminogen Activator Inhibitor (P.A.I.), and tissue Plasminogen Activator (t.P.A.) are the same in venous blood, and peri-lesional arterial blood, in patients undergoing percutaneous transluminal angioplasty for intermittent claudication.

2) To determine the immediate local effects of percutaneous angioplasty on arterial blood rheology, fibrin turnover (as determined by levels of cross-linked FDP's), markers of endothelial damage (plasma vWF), and fibrinolysis (determined by levels of t.P.A. and P.A.I.), in patients with intermittent claudication.

3) To examine the longer-term effects of percutaneous angioplasty on blood rheology and thrombotic mediators in patients with claudication, by comparison of venous blood samples prior to, and 4 months after, percutaneous angioplasty. These changes will be compared with a control group of patients with peripheral arterial disease undergoing arteriography alone.

4) To determine whether or not any relationship exists between pre-angioplasty blood rheology and levels of thrombotic mediators, and the outcome following percutaneous angioplasty.

MATERIALS AND METHODS

Patients & methods

Forty-three (43) patients undergoing percutaneous transluminal angioplasty of symptomatic stenoses (35 cases), or short occlusions (8 cases), in either iliac or femoropopliteal vessels, were recruited. All patients were attending the peripheral vascular surgery unit at Glasgow Royal Infirmary, and underwent a full physical examination prior to entry into the study. Diagnosis of peripheral vascular disease was based on a fall in the ankle-brachial pressure index (ABPI) of more than 0.15, following a standard treadmill exercise test (2km/hr at an incline of 10° until either the onset of symptoms, or a maximum of 200 metres), carried out by myself or a vascular research nurse, and a Seldinger arteriogram showing a stenosis of greater than 50% at a site appropriate to the symptoms. Informed consent for participation in the study was obtained from all patients, and approval obtained from the hospital ethical committee.

Fasting venous blood samples were obtained by myself, with minimal stasis, from the antecubital vein in all patients, 2 to 4 hours prior to angioplasty, and again 16 weeks later at review in the vascular laboratory. The review samples were all taken prior to the patient undergoing a further treadmill exercise test, using the criteria described above, with measurement of ankle-brachial pressure indices (ABPI) prior to and following the test. Samples were obtained, handled, and assayed as described in chapter 2.

All angioplasties were carried out by Dr. A.W. Reid, consultant vascular radiologist, using commercially available angioplasty balloon catheters.

Comparison of venous & arterial blood

In 15 of the 43 patients additional 14ml samples of arterial blood were obtained through a size 5 french angiography catheter, at the site of the arterial lesion (8 downstream of an iliac stenosis, 7 upstream from a superficial femoral lesion), by the radiologist, prior to angioplasty being carried out, and a further sample obtained in the vicinity of the lesion immediately following deflation of the angioplasty balloon. 5mls of each sample of blood were added to tubes containing dried dipotassium EDTA 1.5mg/ml (Monoject, Sherwood Medical, U.K.), for estimation of rheological parameters as previously described, while the remaining 9mls were added to a plastic tube containing 1ml of trisodium citrate (0.109M), and subsequently handled and assayed for cross-linked FDP's, von Willebrand Factor, Plasminogen Activator Inhibitor (P.A.I.) and tissue Plasminogen Activator (t.P.A.), as described in chapter 2.

Controls

In 10 control patients (7 male and 3 female) with angiographically proven peripheral arterial disease and symptoms of intermittent claudication, who were undergoing arteriography by the Seldinger technique, venous blood samples were obtained, stored, and assayed in the manner previously described. The mean age of the control patients was 68 years (range 49-80), and 50% were on antiplatelet therapy pre- and post-angiography. All samples were obtained prior to angiography, and sampling was repeated 12-16 weeks later. At this time clinical examination and measurement of ABPI's was carried out in all patients to ensure that there had been no significant deterioration in the patients peripheral arterial disease following angiography. Samples were subsequently handled and assayed as previously described.

Statistics

All results were stored as described in chapter 2, and all statistical analyses were carried out by myself. Results were compared using non-parametric methods, with pairwise deletion where data values were missing.

RESULTS

Results of percutaneous angioplasty

It was not possible to carry out angioplasty in 1 of the 43 patients due to inability to traverse a short occlusion with a percutaneous guidewire, and this "technical failure" was therefore excluded from further analysis. A further patient was diagnosed as suffering from gastric adenocarcinoma, after a successful PTA but prior to review, and was therefore also excluded.

41 patients therefore completed the study, 24 males and 17 females, with a mean age of 63 years (range 38-77). 25 of these patients had undergone iliac angioplasty, and 16 superficial femoral angioplasty (Table 5.1, p.148). The smoking habits of all the patients in the study remained unchanged over the 4 month follow up period, non-smoking being confirmed by plasma carboxyhaemoglobin estimation (Chapter 2). There were 4 patients who commenced antiplatelet therapy following angioplasty, in addition to 13 patients who remained on antiplatelet therapy throughout the period of the study.

One 70 year old female patient died following surgery for a femoral false aneurysm after successful angioplasty, and in the remaining patients there were 36 cases in which angioplasty improved the ABPI (figures 5.1-5.2, p.149-150), and increased the mean treadmill walking distance from 120 metres (range 40-200m) to 155 metres (range 30-200m), and 4 patients (10%), in whom both clinical and laboratory assessment at 3 months confirmed restenosis or occlusion of the angioplastied lesion (Table 5.1), treadmill distance remaining at a mean level of 94 metres post-angioplasty (97 metres pre-angioplasty). In all 40 of these patients however, radiological assessment immediately post-angioplasty confirmed technical success, while clinical patency was confirmed on examination prior to hospital discharge, 24 hours following the angioplasty.

Comparison of blood rheology measures prior to and 16 weeks after successful angioplasty (Table 5.2, p.151), showed no significant changes in blood rheology following successful percutaneous angioplasty, and a similar stability was observed in the control patients undergoing angiography alone, although there was a significant rise in red cell aggregation observed in control cases (Table 5.3, p.152).

In the control patients there were no significant differences in the levels of any of the potential thrombotic mediators (fibrinogen, FDP's, vWF, Factor VII, t.P.A, and P.A.I.), when pre-angiography and post-angiography results were compared (Table 5.3). Following successful angioplasty there was a significant

	GOOD OUTCOME	RESTENOSIS	DIED	TOTALS
Indication				
Claudication	36	3	1	40
Critical limb ischaemia	1	-	-	1
Site of PTA				
Iliac	**21**	**3**	**1**	**25**
<5cm stenosis	17	1	-	17
>5cm stenosis	4	-	-	4
occlusion <5cm	-	2	1	3
SFA/popliteal	**15**	**1**	-	**16**
<5cm stenosis	8	1	-	9
>5cm stenosis	2	-	-	2
occlusion <5cm	5	-	-	5
Risk Factors				
Continued smoking	23	1	-	24
Ex-smokers	14	2	1	17
Antiplatelet therapy	17	3	-	20
Diabetes Mellitus	2	1	-	3

Table 5.1: Details of 41 percutaneous angioplasty procedures in 41 patients.

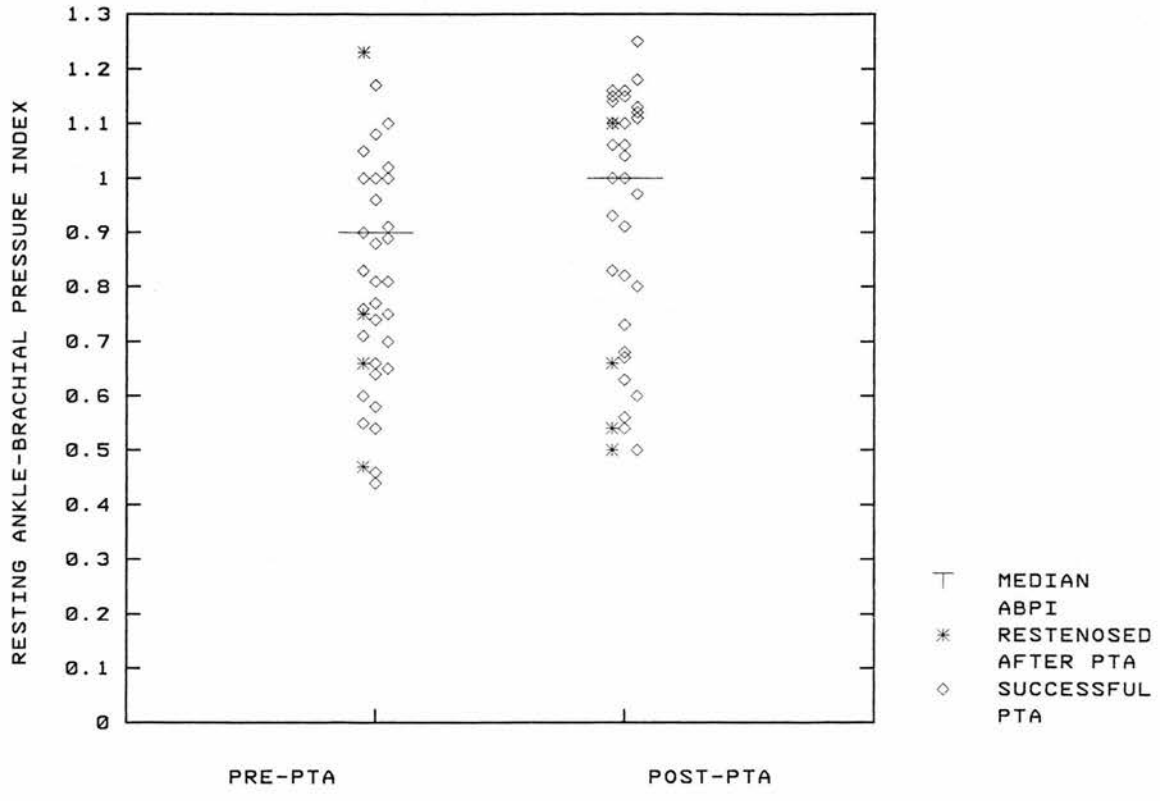


Figure 5.1: Comparison of pre- and post-angioplasty resting ankle brachial pressure index.

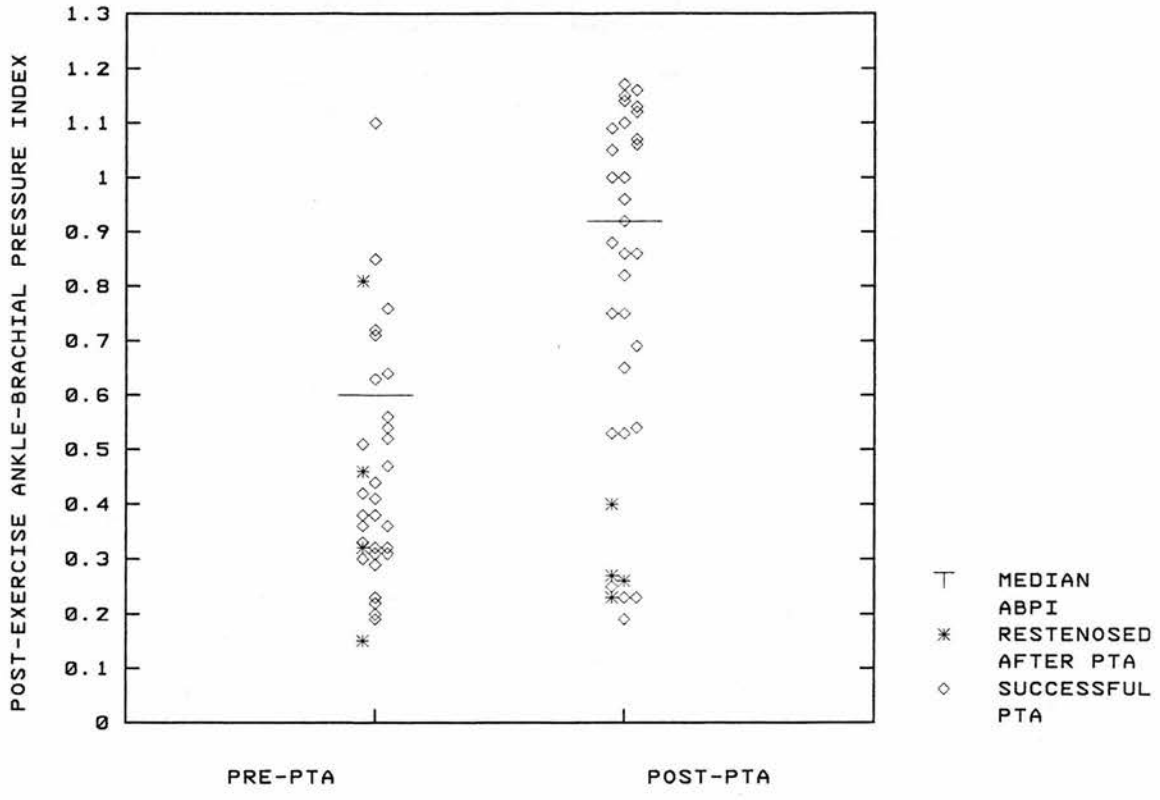


Figure 5.2: Comparison of pre- and post-angioplasty post-exercise ankle brachial pressure index.

VARIABLE	PRE-PTA	POST-PTA	WILCOXON MATCHED PAIRS
fibrin degradation products (ng/ml)	100 (64-134)	140 (99-179)	$p < 0.04$
haematocrit (%)	45 (42-48)	45 (42-48)	$p = 0.06$
Platelet count ($x10^9/l$)	259 (214-287)	269 (226-327)	$p = 0.08$
factor VII (% pool)	120 (104-128)	128 (110-139)	$p = 0.15$
fibrinogen (g/l)	3.5 (2.9-3.8)	3.4 (3.1-4.3)	$p = 0.45$
corrected blood viscosity (mPa.s)	3.30 (3.14-3.36)	3.31 (3.12-3.46)	$p = 0.50$
plasminogen activator inhibitor (% pool)	103 (86-159)	116 (75-159)	$p = 0.46$
White cell Count ($x10^9/l$)	8.6 (6.8-9.8)	8.0 (6.9-9.4)	$p = 0.51$
relative blood viscosity	2.50 (2.38-2.58)	2.44 (2.32-2.54)	$p = 0.52$
von Willebrand factor (iu/dl)	124 (102-157)	124 (93-153)	$p = 0.64$
plasma viscosity (mPa.s)	1.32 (1.28-1.40)	1.36 (1.30-1.39)	$p = 0.66$
tissue plasminogen activator (ng/ml)	7.8 (5.3-10.0)	8.3 (6.0-10.7)	$p = 0.66$
Red cell aggregation (units)	4.2 (3.6-4.9)	4.2 (3.5-5.1)	$p = 0.80$

Table 5.2: Changes in blood rheology and thrombotic mediators following successful percutaneous angioplasty in 36 patients. Figures are median values (interquartile range).

VARIABLE	PRE-ANGIOGRAM	POST-ANGIOGRAM	WILCOXON MATCHED PAIRS
Red cell aggregation (units)	4.6 (3.7-5.4)	5.7 (4.6-6.2)	p = 0.04
Corrected blood viscosity (mPa.s)	3.30 (3.19-3.33)	3.26 (3.04-3.58)	p = 0.12
haematocrit (%)	44 (42-46)	40 (37-46)	p = 0.18
fibrin degradation products (ng/ml)	288 (165-639)	258 (135-592)	p = 0.20
plasma viscosity (mPa.s)	1.37 (1.34-1.44)	1.37 (1.27-1.39)	p = 0.21
factor VII (% pool)	113 (101-128)	118 (98-130)	p = 0.34
fibrinogen (g/l)	4.0 (3.5-4.4)	3.7 (3.5-4.6)	p = 0.39
Platelet count ($x10^9$/l)	248 (234-305)	240 (233-287)	p = 0.58
White cell Count ($x10^9$/l)	8.5 (6.4-9.4)	8.2 (7.0-9.6)	p = 0.58
relative blood viscosity	2.40 (2.33-2.48)	2.47 (2.35-2.63)	p = 0.78
tissue plasminogen activator (ng/ml)	8.8 (6.5-9.5)	8.0 (6.9-9.6)	p = 0.80
von Willebrand factor (iu/dl)	124 (93-162)	121 (107-132)	p = 0.91
plasminogen activator inhibitor (% pool)	119 (87-136)	114 (84-122)	p = 1.00

Table 5.3: Blood rheology and thrombotic mediators pre and post-angiography in 10 control patients. Figures are medians (interquartile range).

increase in fibrin turnover, as determined by levels of cross-linked fibrin degradation products, when compared with pre-operative levels ($p < 0.04$). Levels of other variables measured were similar both pre- and post-angioplasty (Table 5.2) in these patients.

Comparison of pre-angioplasty blood rheology and levels of thrombotic mediators in the 2 outcome groups (successful PTA versus restenosis/occlusion) failed to reveal any significant differences in the 2 outcome groups (Table 5.4, p.154), although on comparison of pre- and post-operative FDP levels it appears that successful angioplasty is associated with alterations in fibrin turnover that are not observed in cases where angioplasty fails (Fig. 5.3, p.156). However as there were only 4 failures these conclusions must be limited.

Rheology & thrombotic mediators in arterial & venous blood

Arterial and venous samples were available for comparison in 15 patients (mean age 63 years, range 38-75 yrs.), 10 male and 5 female. In 2 of these cases there was evidence of significant haemodilution (presumably as a result of flushing the angiography catheter with heparinised saline), and the intra-arterial samples from these patients were therefore excluded from subsequent analyses. One additional sample was found to contain clots, and was also discarded, leaving 12 samples for comparison of arterial and venous levels of blood rheology, cross-linked FDP's, von Willebrand factor, t.P.A., and P.A.I. 6 of these arterial samples were obtained downstream of an iliac lesion, and 6 upstream of a superficial femoral lesion.

Arterial blood rheology was almost identical to venous blood rheology in all samples, with no significant differences in any of the parameters, and both cross-linked FDP levels, and P.A.I. levels were also similar (Table 5.5, p.157). von Willebrand factor levels (Figure 5.4, p.158), and tissue Plasminogen Activator (t.P.A.) levels (Figure 5.5, p.159) were however significantly lower in arterial blood than in venous blood, although correlations between arterial and venous levels were above 0.5 for all measurements except vWF and relative blood viscosity (Table 5.5).

Changes in arterial blood after angioplasty

Pre- and post-angioplasty arterial samples were compared in 12 of the 15 patients, 3 patients being excluded on the grounds of differences in pre- and post-angioplasty haematocrit suggesting haemodilution (2 cases), or due to a clotted citrate sample (1 case).

VARIABLE	SUCCESSFUL PTA	FAILED PTA	MANN WHITNEY U-TEST
haematocrit (%)	45 (42-48)	41 (40-41)	p = 0.10
von Willebrand factor (iu/dl)	124 (102-157)	179 (135-179)	p = 0.14
relative blood viscosity	2.50 (2.38-2.58)	2.35 (2.31-2.39)	p = 0.18
plasma viscosity (mPa.s)	1.32 (1.28-1.40)	1.40 (1.39-1.42)	p = 0.23
Platelet count ($x10^9/l$)	259 (214-287)	315 (248-315)	p = 0.25
fibrin degradation products (ng/ml)	100 (64-134)	127 (108-127)	p = 0.39
White cell Count ($x10^9/l$)	8.6 (6.8-9.8)	9.2 (7.6-9.2)	p = 0.43
tissue plasminogen activator (ng/ml)	7.8 (5.3-10.0)	3.9 (3.7-14.5)	p = 0.55
factor VII (% pool)	120 (104-128)	137 (128-148)	p = 0.56
Red cell aggregation (units)	4.2 (3.6-4.9)	4.7 (3.2-4.7)	p = 0.65
fibrinogen (g/l)	3.5 (2.9-3.8)	3.6 (3.1-3.6)	p = 0.70
corrected blood viscosity (mPa.s)	3.30 (3.14-3.36)	3.30 (3.28-3.32)	p = 0.85
plasminogen activator inhibitor (% pool)	103 (86-159)	96 (58-201)	p = 0.85

Table 5.4: Comparison of levels of baseline rheology and thrombotic mediators in successful (36 cases) and failed (4 cases) percutaneous transluminal angioplasty. Figures are median (interquartile range).

In these cases there were no significant changes in arterial blood rheology or Plasminogen Activator Inhibitor (P.A.I.) levels immediately following angioplasty (Table 5.6, p.160). There was a trend towards a significant rise in the levels of cross-linked FDP's immediately following angioplasty (Figure 5.6, p.161), that was more apparent in venous samples taken 16 weeks after angioplasty (Fig. 5.7, p.162), together with a significant rise in vWF levels (Figure 5.8, p.163), although this was not maintained in the post-angioplasty venous samples (Fig. 5.9, p.164). There was also a significant fall in tissue Plasminogen Activator (t.P.A.) levels (Figure 5.10, p.165) in arterial blood immediately following angioplasty, although this was not apparent in venous samples 16 weeks later (Fig. 5.11, p.166).

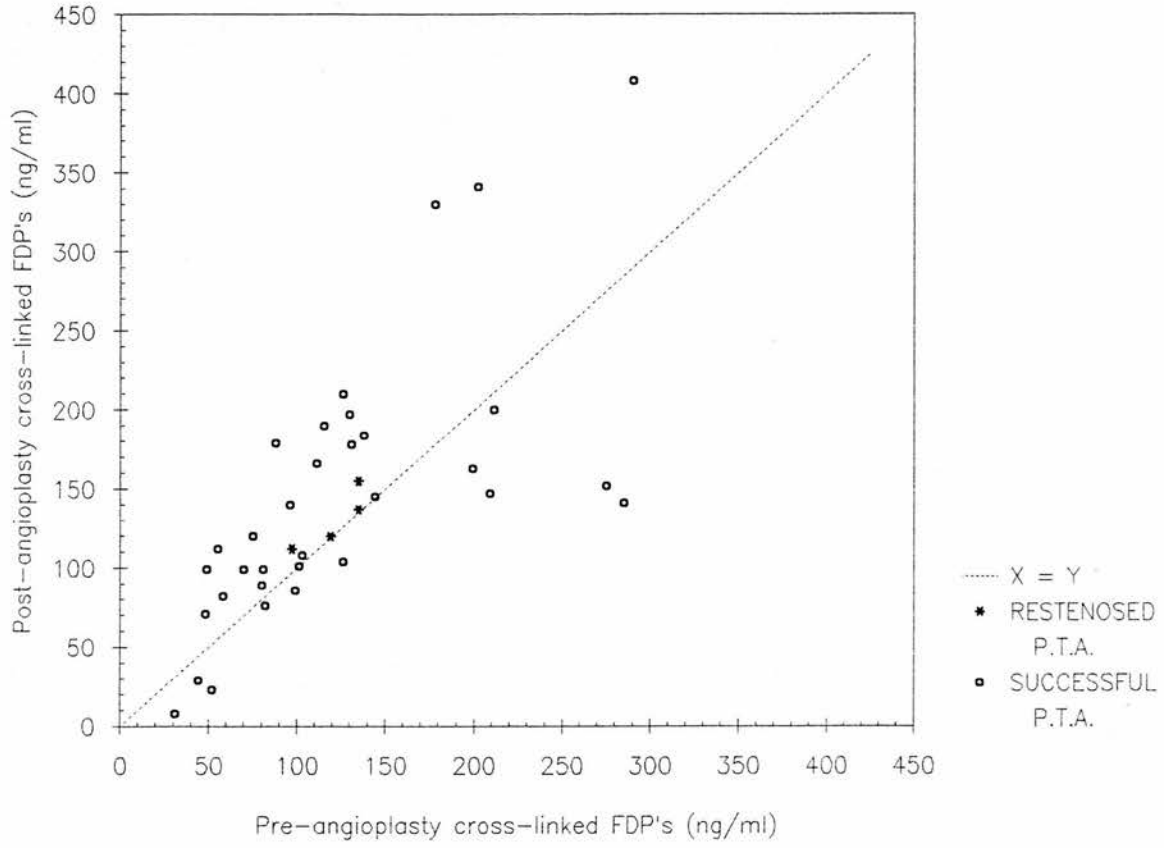


Figure 5.3: Plot of pre-angioplasty fibrin degradation product level against post-angioplasty levels in 40 patients undergoing percutaneous angioplasty.

VARIABLE	VENOUS BLOOD	ARTERIAL BLOOD	WILCOXON MATCHED PAIRS	R VALUE
von Willebrand factor (iu/dl)	126 (114-142)	68 (56-102)	p = 0.003	R = 0.36
tissue plasminogen activator (ng/ml)	7.5 (5.5-10.0)	6.7 (4.5-7.5)	p = 0.01	R = 0.90
haematocrit (%)	45 (42-46)	44 (43-46)	p = 0.22	R = 0.96
plasminogen activator inhibitor (% pool)	96 (72-159)	95 (74-146)	p = 0.24	R = 0.81
relative blood viscosity	2.52 (2.41-2.64)	2.41 (2.39-2.62)	p = 0.37	R = 0.45
plasma viscosity (mPa.s)	1.30 (1.27-1.39)	1.28 (1.24-1.38)	p = 0.50	R = 0.78
corrected blood viscosity (mPa.s)	3.31 (3.21-3.59)	3.27 (3.06-3.34)	p = 0.53	R = 0.48
fibrin degradation products (ng/ml)	106 (76-120)	100 (79-149)	p = 0.59	R = 0.79
Red cell aggregation (units)	4.4 (3.4-5.0)	4.4 (2.3-5.0)	p = 1.00	R = 0.60

Table 5.5: Comparison of blood rheology and thrombotic mediators in venous and peri-lesional arterial blood, in 12 patients undergoing PTA. Figures are medians (interquartile range). Spearman rank order correlation values are also shown.

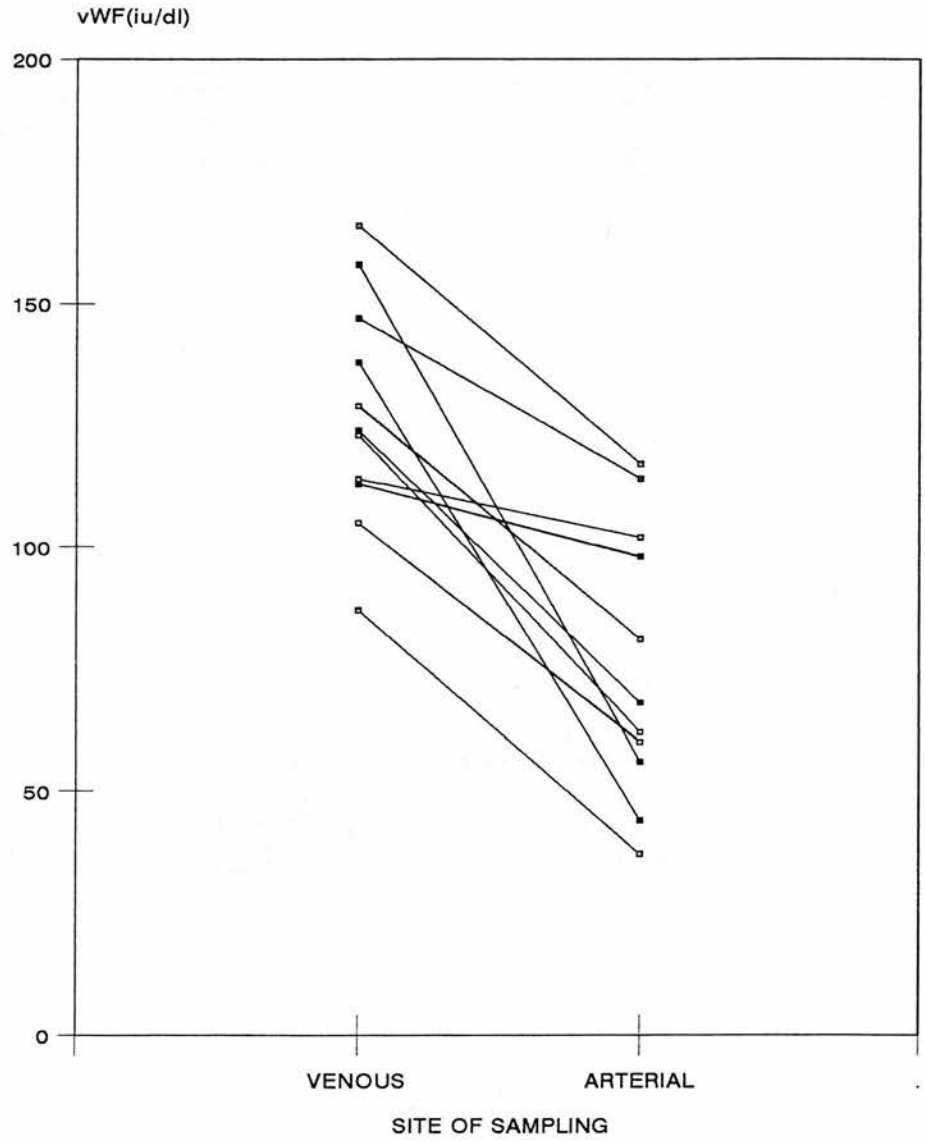


Figure 5.4: Arterial and venous levels of von Willebrand Factor (vWF) in 11 patients undergoing PTA. p = 0.003, Wilcoxon matched pairs test.

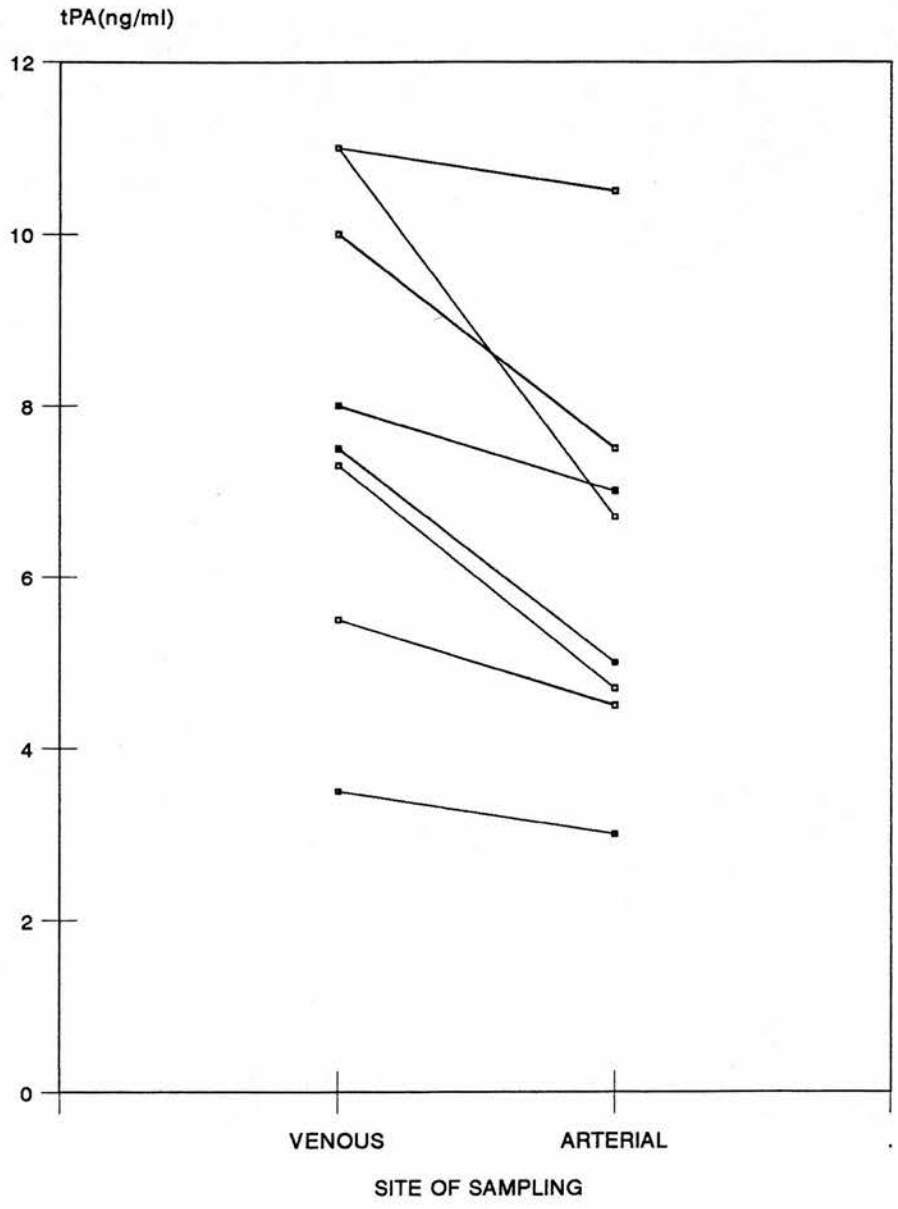


Figure 5.5: Arterial and venous levels of tissue Plasminogen Activator (t.P.A.) in 8 patients undergoing PTA. p = 0.01, Wilcoxon matched pairs test.

VARIABLE	PRE-PTA	POST-PTA	WILCOXON MATCHED PAIRS
von Willebrand factor (iu/dl)	68 (58-100)	88 (74-132)	p = 0.01
tissue plasminogen activator (g/l)	6.9 (4.8-7.8)	4.5 (2.9-5.4)	p = 0.02
fibrin degradation products (ng/ml)	100 (81-174)	108 (90-154)	p = 0.06
relative blood viscosity	2.40 (2.34-2.42)	2.49 (2.37-2.55)	p = 0.13
plasma viscosity (mPa.s)	1.29 (1.26-1.37)	1.28 (1.24-1.34)	p = 0.14
Red cell aggregation (units)	4.4 (3.6-5.0)	2.6 (1.9-4.1)	p = 0.34
haematocrit (%)	44 (42-44)	44 (41-44)	p = 0.40
plasminogen activator inhibitor (% pool)	80 (60-88)	76 (63-86)	p = 0.64
corrected blood viscosity (mPa.s)	3.20 (3.02-3.28)	3.27 (3.02-3.31)	p = 0.84

Table 5.6: Comparison of blood rheology and thrombotic mediators in pre and post-angioplasty arterial blood, in 12 patients undergoing PTA. Figures are medians (interquartile range).

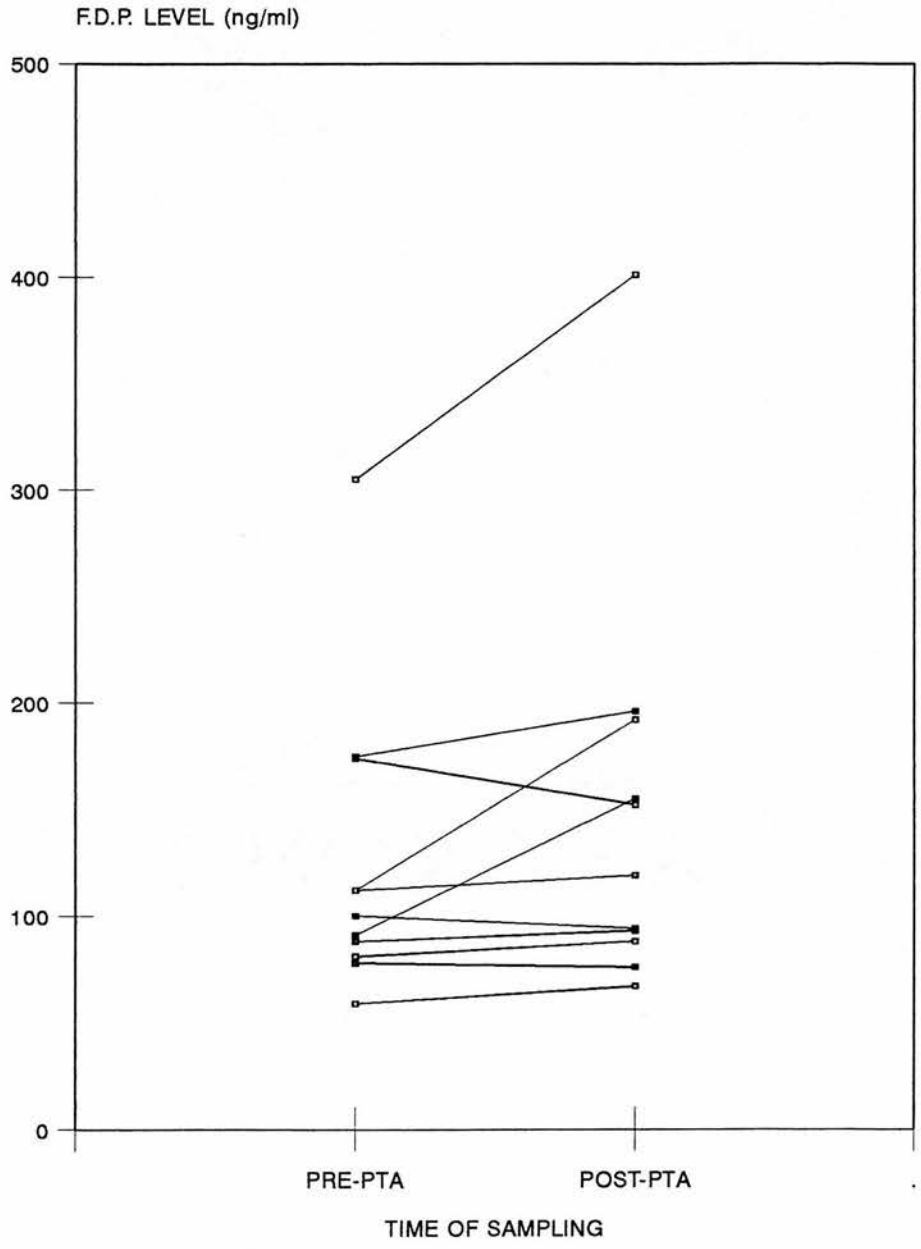


Figure 5.6: Pre- and post-angioplasty levels of cross-linked fibrin degradation products in arterial blood in 11 patients undergoing PTA. $p = 0.06$, Wilcoxon matched pairs test.

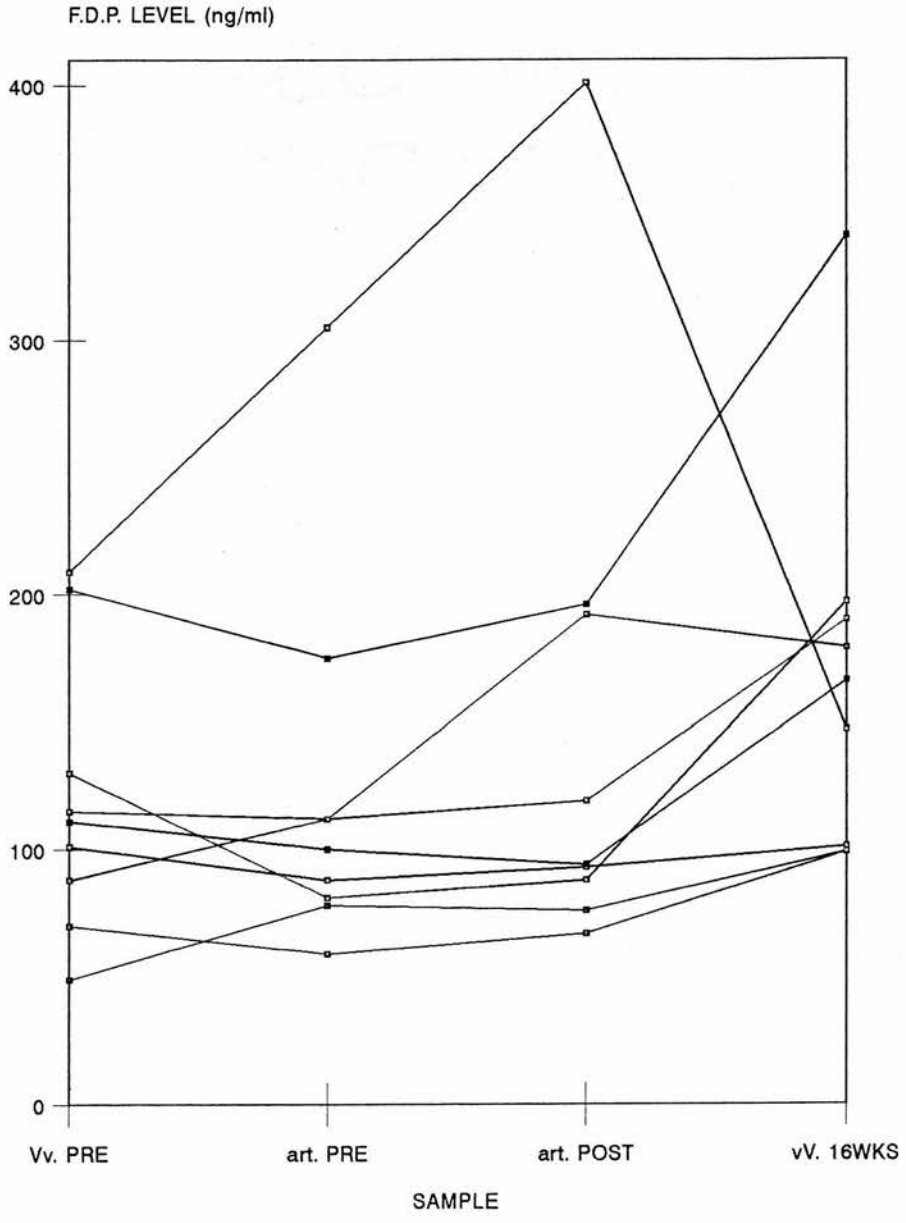


Figure 5.7: Serial changes following angioplasty: Plasma fibrin degradation product levels in 9 cases (Vv. = venous blood, art. = arterial, Pre = pre-angioplasty, post = post-angioplasty).

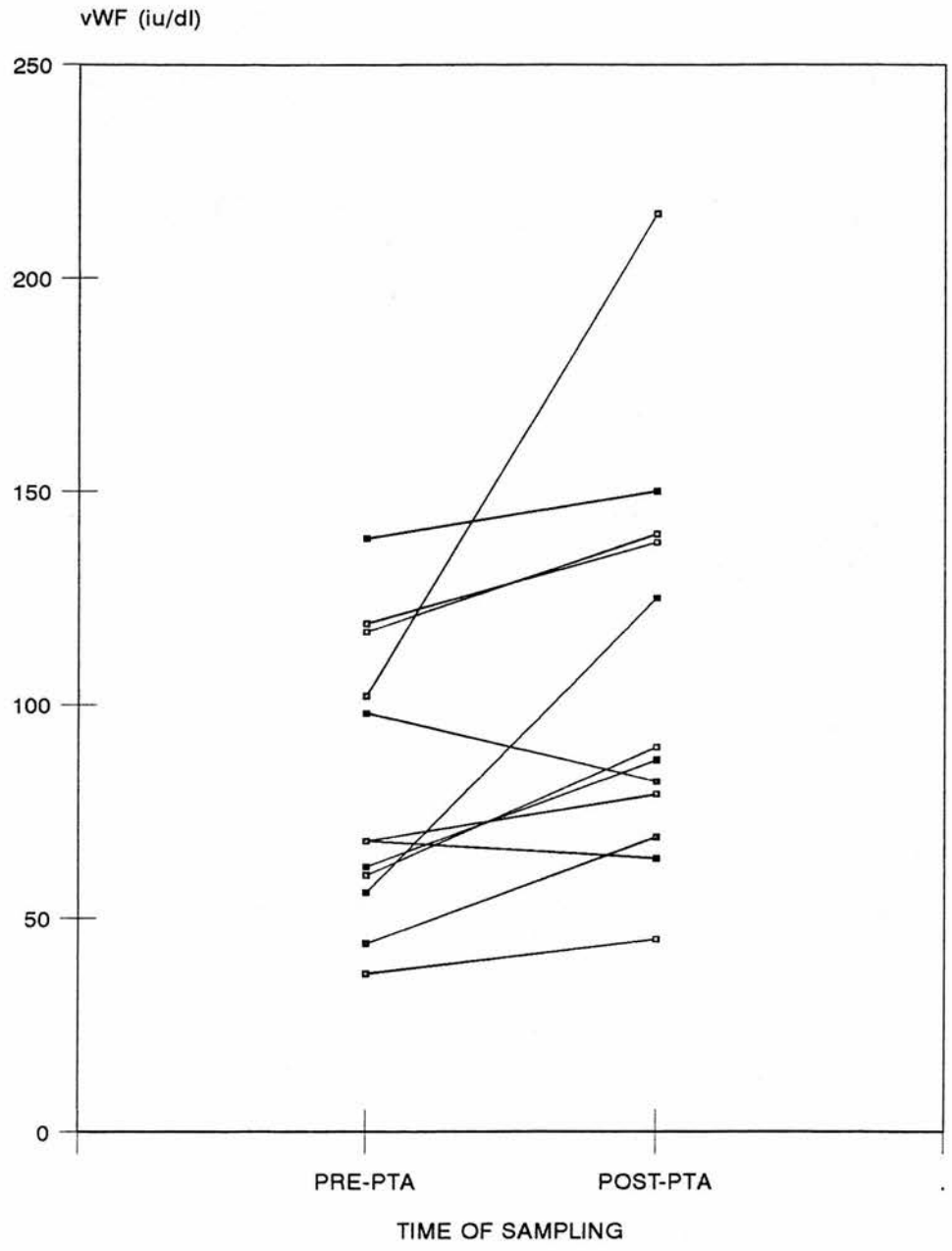


Figure 5.8: Pre- and post-angioplasty levels of von Willebrand Factor (vWF) in arterial blood in 12 patients undergoing PTA. p = 0.01, Wilcoxon matched pairs test.

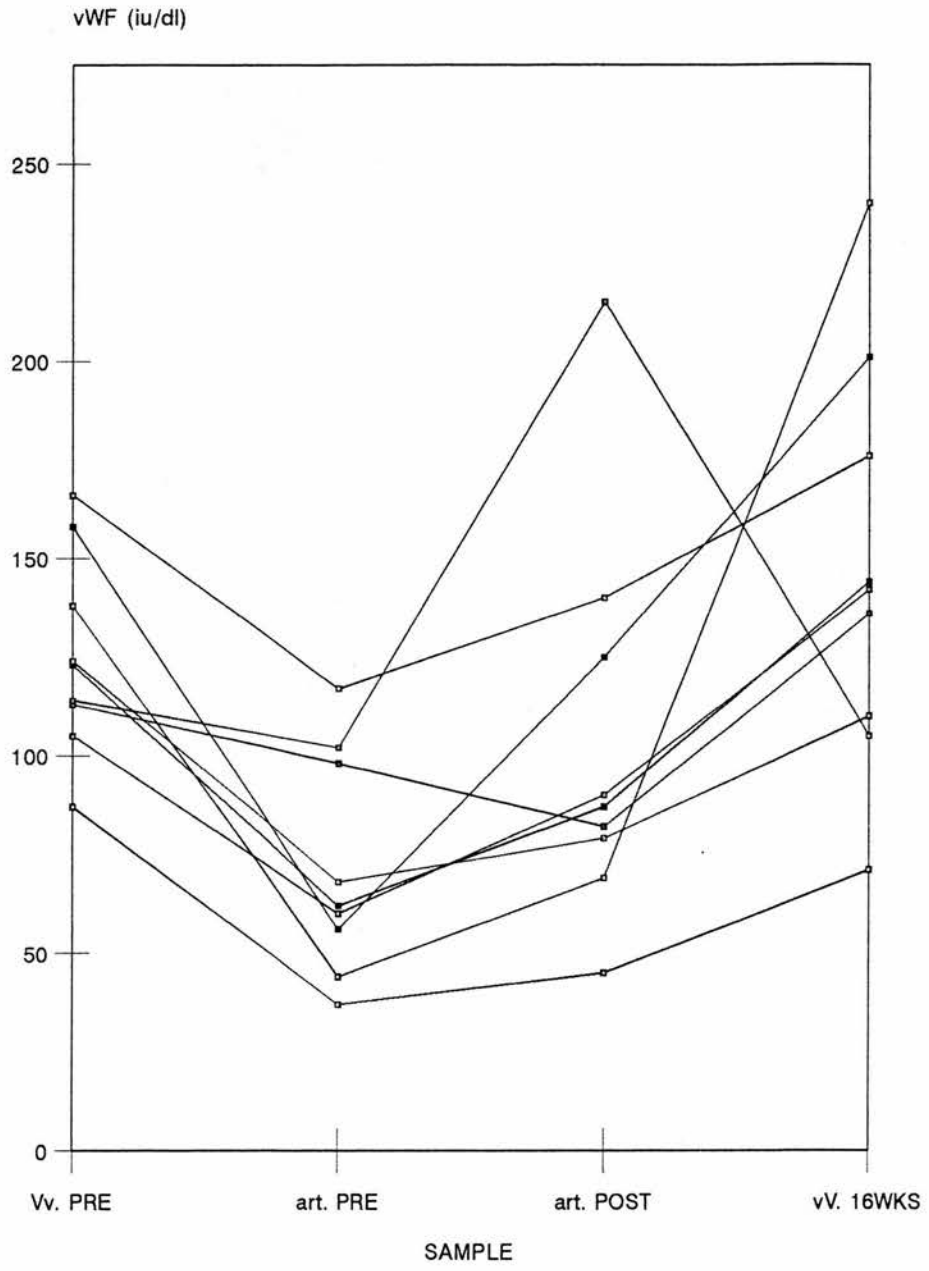


Figure 5.9: Serial changes following angioplasty: Plasma von Willebrand Factor levels in 9 cases (Vv. = venous blood, art. = arterial, Pre = pre-angioplasty, post = post-angioplasty).

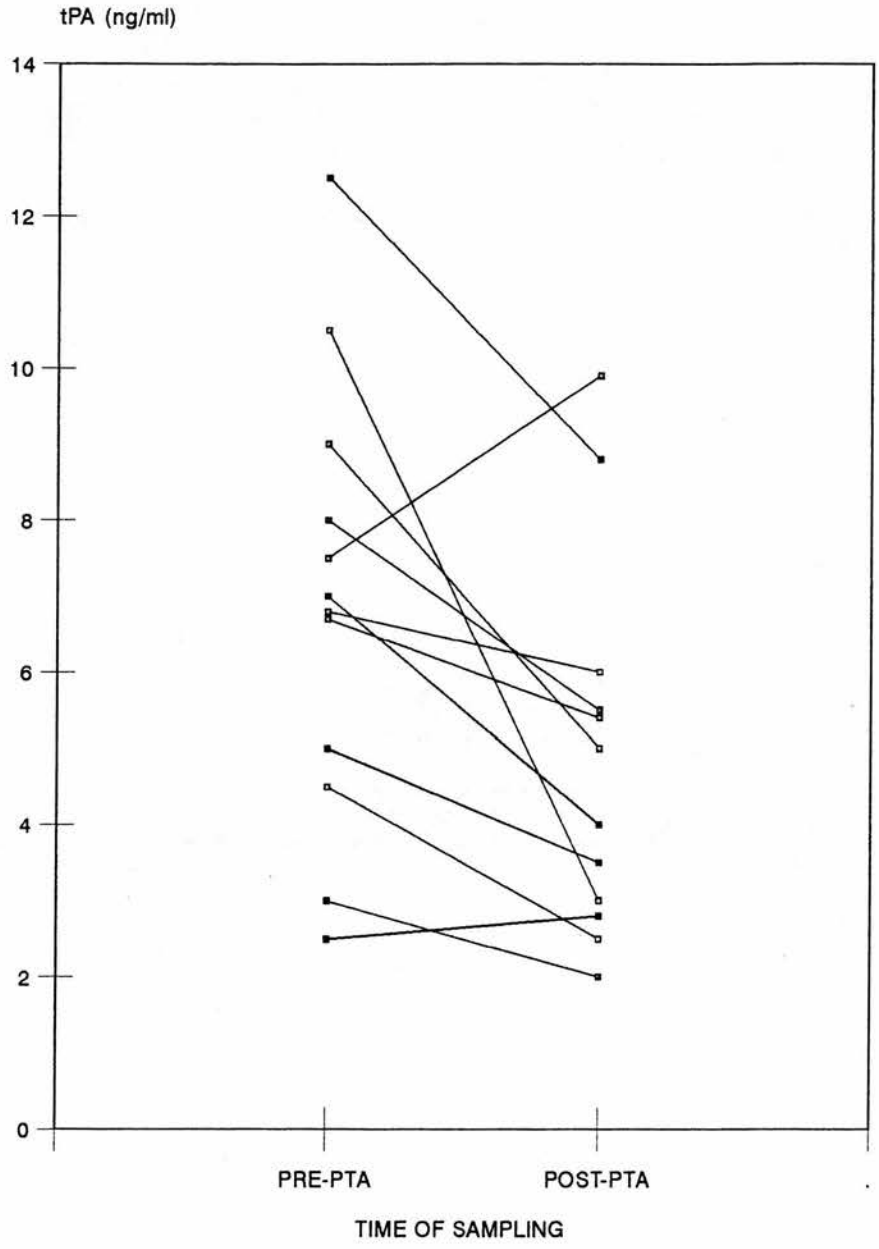


Figure 5.10: Pre- and post-angioplasty levels of tissue Plasminogen Activator (t.P.A.) in arterial blood in 12 patients undergoing PTA. $p = 0.02$, Wilcoxon matched pairs test.

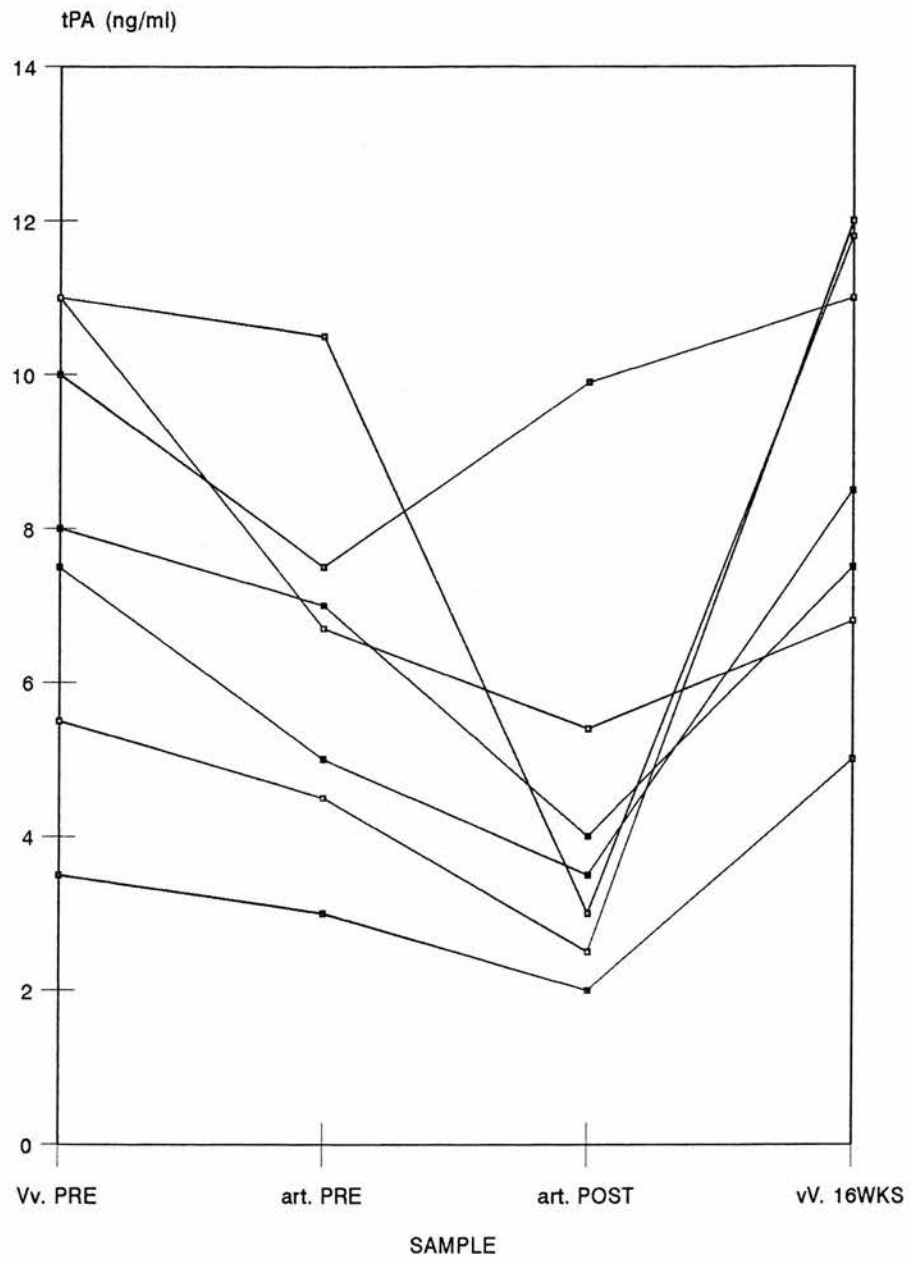


Figure 5.11: Serial changes following angioplasty: Tissue Plasminogen Activator levels in 9 cases (Vv. = venous blood, art. = arterial, Pre = pre-angioplasty, post = post-angioplasty).

DISCUSSION

The patients described in this study were not randomly selected but are a representative sample of cases selected for angioplasty at one vascular surgery centre. 90% of the patients had both clinical and Doppler evidence of successful angioplasty at 4 months, in keeping with other reports (Cambria et al, 1987, Michaels, 1990), although when a diagnosis of failure is made on the basis of non-invasive vascular laboratory studies, rather than on angiographic appearances, it is impossible to be certain that symptoms are attributable to restenosis of the angioplastied lesion, rather than arising from other significant arterial lesions. In this series failure was more common in iliac lesions (Table 5.1), in which angioplasty is recognised to have a lower success rate (Al-Kutoubi, 1992).

The relatively low early failure rate of angioplasty in this series, coupled with the previously expressed doubts about the source of recurrent symptoms in the patients studied, makes it difficult to draw any definite conclusions on the role of blood rheology and thrombotic mediators in restenosis following angioplasty. To address this issue in appropriate detail will require large numbers of patients and invasive angiographic follow up. However this was not the primary aim of this study, which has concentrated on the effects of successful angioplasty on blood rheology and thrombotic parameters, as the success of angioplasty can be assessed non-invasively by improvement in ABPI and walking distance.

Arterial & venous comparisons

Altered blood viscosity in patients with peripheral arterial disease, has been demonstrated in a number of studies (Dormandy et al, 1973A, Lowe, 1986), and it has been suggested that local alterations in rheological parameters may have a role in the distribution of the lesions of atherosclerosis (Schwartz et al, 1989). Pathological studies in patients with peripheral arterial disease have implicated fibrin deposition in the development of the arterial lesions (Duguid, 1949, Smith & Staples, 1981), and in addition there is evidence implicating increased fibrin turnover in the disease process, elevated levels of cross-linked fibrin degradation products (FDP's) having been demonstrated in patients with symptomatic peripheral arterial disease (Reid, 1991, Al-Zahrani et al, 1992).

These findings have however all been made in venous blood samples, taken from the antecubital vein, and in view of the focal nature of the lesions of atherosclerosis, the situation in arterial blood may be different. These results however indicate that venous sampling represents an accurate reflection of the

composition of arterial blood in patients with symptomatic peripheral arterial disease, with good correlations between arterial and venous levels of most mediators. The major exception to this observation is with respect to von Willebrand factor levels, which, despite a poor correlation between arterial and venous levels, appear to be consistently lower in arterial blood. The reason for this difference is not known, but may reflect decreased production from a damaged arterial endothelium at sites of stenosis, or binding of vWF to exposed subendothelium at these sites.

In addition, tissue Plasminogen Activator (t.P.A.) levels are significantly lower in peri-lesional arterial blood than in venous blood, although complete data was only available in 8 cases. This may also be due to impaired production of t.P.A. by damaged endothelium in the vicinity of arterial stenoses, as t.P.A. is mainly synthesised by endothelial cells (Hashimoto et al, 1987). However levels of P.A.I., also synthesised by endothelial cells (Kruithof, 1988), would be expected to be similarly reduced in this situation, but this was not observed. The implication of this is that the equilibrium between t.P.A. and P.A.I. in peri-lesional arterial blood is not the same as it is in venous blood, with the balance being shifted in favour of a reduction in fibrinolytic activity in peri-lesional arterial blood. This may be a contributory factor in the development of atherosclerotic plaques and arterial stenosis, which have been shown to contain fibrin (Duguid, 1949, Smith & Staples, 1981).

The similarity between arterial and venous levels of cross-linked FDP levels indicates that any contribution that altered fibrin turnover makes to peripheral arterial disease, and in particular to the progression of atherosclerotic plaques (Duguid, 1949, Smith & Staples, 1981), is not made by local alterations in fibrin turnover at the sites of development of symptomatic stenoses. The elevated levels of FDP that have been documented in peripheral arterial disease (Reid, 1991, Al-Zahrani et al, 1992, Peltonen et al, 1992)), appear therefore to reflect a systemic alteration in fibrin turnover.

These results do little to support any suggestion of significant local alterations in haemostatic and rheological parameters at the sites of symptomatic lesions in patients with peripheral arterial disease, and confirm that venous blood sampling provides an accurate reflection of the composition of arterial blood in such patients, although it consistently overestimates the concentration of the endothelial products vWF and t.P.A. present in arterial blood samples taken from the vicinity of a symptomatic stenosis.

Effects of angioplasty on arterial & venous blood

The stability of arterial rheological parameters immediately following angioplasty is unremarkable: the changes in blood rheology associated with peripheral arterial disease occur in association with chronic arterial insufficiency, and are partly due to a chronic increase in inflammatory products such as fibrinogen (Reid, 1991). In the absence of inflammatory changes arterial rheology will remain unchanged in the early post-angioplasty period.

The observed rise in vWF immediately following angioplasty is in keeping with the endothelial damage produced by balloon angioplasty. This consists of cell injury and loss (Block et al, 1980, 1981), brought about by splitting of the intima, and medial stretching, that accompanies inflation of the angioplasty balloon (Castenada-Zuniga et al, 1980), presumably resulting in release of vWF at the site of vascular injury (Wagner & Bonfanti, 1991). This release of vWF appears to be a transient phenomenon however, as venous levels in the same patients are unchanged from their pre-angioplasty levels 4 months later, and remain unchanged pre-and post-angioplasty in the larger group of patients. Analysis of pre-operative vWF levels by outcome, suggests that there is a tendency for angioplasty to fail in patients with higher initial vWF levels. This may indicate that failures occur in those patients with the most severe disease, as vWF levels are higher in more severe disease (Chapter 3), however in view of the small number of cases involved it is not possible to draw any meaningful conclusions from this observation.

The fall in t.P.A. levels immediately following angioplasty is not maintained in venous blood 4 months later, and may be a transient local response to fibrin formation at the site of arterial injury. Prior animal models of arterial injury have demonstrated a similar fall in t.P.A. after arterial injury (Clowes et al, 1990). Tissue-type Plasminogen activator (t.P.A.) binds to fibrin, significantly improving its catalytic efficiency (Kruithof, 1988), and in this way accelerating the lysis of fibrin. It is presumably this binding of t.P.A. to fibrin deposited at the angioplasty site (Block et al, 1980) that results in the observed fall in t.P.A. levels in arterial blood immediately following angioplasty.

This binding of t.P.A. to fibrin stimulates plasmin production (Kruithof, 1988), leading to fibrinolysis, which may account for the small increase in arterial cross-linked FDP levels observed following angioplasty. This rise in FDP's following angioplasty was only minimal, however, perhaps because the post-angioplasty arterial sampling was performed immediately following the procedure, while local fibrin turnover may increase gradually after angioplasty. This belief is

supported by the observation that 16 weeks after angioplasty, FDP's are elevated further from their pre-angioplasty level.

An increase in the lysis of cross-linked fibrin is probably an essential response to the fibrin deposition that has been demonstrated to occur at sites of angioplasty (Block et al, 1980) if arterial occlusion and restenosis is to be avoided, and this study suggests that following successful angioplasty, in addition to the rise in arterial FDP's immediately following angioplasty, there is a sustained rise in venous FDP's that persists for at least 4 months. Closer examination of pre- and post-angioplasty levels of FDP's however, confirms that the rise in FDP's following successful PTA is not a uniform observation, and a number of cases demonstrate a reduction in FDP levels following successful angioplasty.

It is not possible to interpret the observation that cases of restenosis show minimal alteration in fibrin turnover after PTA because the numbers involved are too small, and longer follow-up of a larger number of cases would be required to further investigate this. It is however conceivable that FDP's rise following angioplasty as a consequence of an increase in fibrin turnover at the site of angioplasty, secondary to residual arterial damage and delayed restoration of endothelial cover. In these circumstances an increase in fibrin turnover would be required to maintain patency of the damaged arterial segment. It has previously been observed that incomplete restoration of the arterial lumen following angioplasty results in high shear deposition of platelets at the site of angioplasty (Chesebro et al, 1987). This leads to formation of platelet-fibrin thrombi, which can lead to restenosis and occlusion (Faxon et al, 1987. Harker, 1987). Lysis of such platelet-fibrin thrombi (resulting in an increase in FDP's) would however reduce the likelihood of restenosis and occlusion in such a situation.

When, in contrast, fibrin turnover decreases markedly in the 4 months following angioplasty, this may be because angioplasty has completely restored the arterial lumen with minimal arterial damage, and consequently rapid endothelial regeneration, leading to a reduction in fibrin turnover and FDP levels.

It is also possible that the rise in FDP's observed following angioplasty is the result of worsening of arterial disease in the 4 months following angioplasty, as there is a strong association between the severity of arterial disease, and FDP levels in patients with symptomatic peripheral vascular disease (Reid, 1991, Woodburn et al, 1993A). The effect of angioplasty on the progression of arterial disease has not yet been studied, but there is some animal work indicating that disease progression is more rapid distal to a 50% stenosis, than it is to more severe stenoses (Bomberger et al, 1981).

Because angioplasty often fails to fully restore the arterial lumen to its original diameter, with the original plaque remaining while the non-diseased portion of the arterial wall is stretched to widen the lumen (Zarins et al, 1982), the residual effect of angioplasty is often to leave a minor degree of stenosis present in the recanalised vessel. The presence of these minimal residual stenoses may accelerate disease progression downstream of the site of angioplasty, with consequent increased fibrin turnover and incorporation into plaques (Smith et al, 1992) reflected by an increase in FDP levels.

The lack of any significant alterations in thrombotic mediators in patients undergoing angiography alone confirms the fact that the observations made in this study are a consequence of undertaking percutaneous angioplasty in patients with peripheral arterial disease. The observations made confirm that percutaneous angioplasty has little effect on many thrombotic and rheological parameters associated with peripheral arterial disease, an observation that may in part be due to the fact that patients undergoing angioplasty have less extensive disease than patients undergoing surgery. However the observation that fibrin turnover (as reflected by levels of cross-linked FDP's) is significantly increased immediately after angioplasty, and that this increase is maintained for at least 4 months following the procedure, merits further investigation, as this increase in FDP's may be associated with an increase in the severity of peripheral arterial disease.

Summary

These studies have demonstrated that the measurement of venous blood rheology and most thrombotic mediators show a strong correlation with, and provide an accurate reflection of, peri-lesional arterial values, in patients with symptomatic peripheral arterial disease. Investigators should however bear in mind that venous levels of von Willebrand Factor antigen are significantly higher than peri-lesional arterial levels, and that there is only a weak correlation between the venous and arterial levels.

Percutaneous transluminal angioplasty produces transient alterations in a number of potential thrombotic mediators, that are probably a direct effect of the arterial injury produced by the procedure, but these soon return to baseline levels with the exception of fibrin turnover, as determined by crosss-linked FDP levels, which remain altered for at least 4 months following angioplasty. The magnitude and nature of these alterations may be related to progression of arterial disease following angioplasty and further studies are required to investigate these observations.

These findings are of particular importance in view of the expansion in the number of patients undergoing angioplasty, a procedure which has never been subjected to any rigourous investigation of its long term effects, and the trend towards offering angioplasty to those patients with mild claudication who would previously have been treated by conservative measures alone. Further long term studies of the biochemical effects of angioplasty are required to identify aspects of the long-term effects of the procedure that may be appropriate targets for pharmacological manipulation.

CHAPTER 6

Blood rheology, thrombotic mediators, and the outcome following infra-inguinal bypass grafting

INTRODUCTION

Symptomatic peripheral arterial disease commonly results from superficial femoral artery disease (Bloor, 1961), with surgical bypass of occluded superficial femoral arteries being required to relieve claudication and critical limb ischaemia. While the results of proximal revascularisation procedures are excellent (Reid & Pollock, 1991), the outcome following femoropopliteal and femoro-distal grafting is less encouraging (Whittemore et al, 1990, McCollum et al, 1991B).

There are a number of factors that affect the outcome of infra-inguinal bypass grafting, including the quality of run-off vessels, the graft material utilised (Rutherford, 1990, Tordoir et al, 1993) and the diameter and quality of the bypass conduit (Wengerter et al, 1990). Intrinsic patient hypercoagulability may also contribute to graft failure (Rutherford, 1990). Alterations in blood rheology and levels of potential thrombotic mediators in patients with peripheral arterial disease, may increase thrombogenicity and therefore influence the outcome following revascularisation surgery.

Elevations in blood viscosity have been shown to reduce blood flow along vessels (Dormandy, 1971), while elevated plasma fibrinogen 6 months *after* surgery predicts femoropopliteal vein graft occlusion (Wiseman et al, 1989). Animal studies have indicated that plasma von Willebrand factor is an essential requirement for the development of occlusive thrombi at sites of arterial injury (Brinkhous et al, 1991), and that low levels of vWF protect against the development of atherosclerosis (Badimon & Fuster, 1992). There are therefore a number of potential roles for these and other thrombotic mediators in infra-inguinal bypass graft failure.

Altered levels of thrombotic mediators are of particular interest because they may be more amenable to pharmacological manipulation than the other factors contributing to graft failure. Any relationship between potential thrombotic mediators and the outcome following infra-inguinal bypass grafting offers a means of improving pre-operative patient selection for such surgery, and identifying situations where adjuvant pharmacological therapy may improve the results of surgery.

Although infra-inguinal graft occlusion is readily detected by clinical means, identification of the failing graft, in which intervention may prevent occlusion, is of greater importance (DeWeese & Green, 1990). Colour duplex scanning and other non-invasive vascular laboratory techniques (Wyatt et al, 1991, Idu et al, 1992), offer alternatives to angiography for the early identification of

"at risk" infra-inguinal grafts (Harris, 1991, Renton et al, 1991, Hatsukami, 1992, Davies et al, 1993), and the use of these non-invasive techniques in a graft surveillance program enables accurate assessment of the results of revascularisation surgery. This information can be used to relate clinical events to any post-operative changes that may occur in rheological and thrombotic parameters. The haemorheological responses to infra-inguinal grafting have not been previously reported.

Aims:

1) To develop a predictive index for poor outcome (death or graft occlusion) following infra-inguinal bypass grafting, based on pre-operative blood rheology, levels of thrombotic mediators, and clinical and radiological information.

2) To establish, by means of serial measurements, the effects of successful infra-inguinal revascularisation surgery on the abnormal blood rheology and levels of potential thrombotic mediators previously observed in patients with peripheral arterial disease, and to investigate the effects of the material used for bypass grafting on these responses.

3) To determine the pattern of post-operative changes in blood rheology and thrombotic mediators in vein grafts developing stenoses, and to compare these changes with the changes occurring in vein grafts remaining free of stenoses.

4) To investigate the value of non-invasive imaging and assessment techniques in identifying infra-inguinal grafts that require further assessment by angiography.

MATERIALS AND METHODS

Patients

186 consecutive infra-inguinal bypass grafts in 165 patients attending the Unit for Peripheral Vascular Surgery, Glasgow Royal Infirmary, and the Vascular Surgery Unit, Gartnavel General Hospital, were recruited into this study prior to undergoing surgery, and followed up for up to 1 year post-operatively. Venous blood samples were obtained without stasis prior to surgery, and at 3, 6, and 12 months post-operatively, or until the graft was shown to be occluded, whichever was the shorter period. A full history and examination was obtained from each

patient, and informed consent for participation in the study was obtained. The study was approved by the relevant hospital's ethical committee.

On post-operative visits graft status was assessed by clinical examination in addition to the vascular laboratory methods described below, and the time of graft occlusion determined by clinical history. Patient mortality was confirmed from hospital records, and the patients' own general practitioner where appropriate.

Graft surveillance

All patients whose grafts were patent at discharge from hospital attended for graft surveillance at 3, 6, and 12 months post-operatively. Patients attending Gartnavel General Hospital underwent clinical examination and measurement of resting ankle-brachial pressure indices at each visit, as described in chapter 2, and colour duplex scanning of the graft was carried out by myself in conjunction with Mrs. Roz Carter, Vascular Laboratory Technician, using an Acuson 128 Scanner (Acuson Corporation (U.K.), Stevenage, Herts.). Duplex scans were performed at each surveillance visit, and in grafts where the duplex scan was suggestive of significant stenosis, or where symptoms of claudication or rest pain had returned, patients underwent arteriography for definitive diagnosis. Significant stenosis on colour duplex scanning was based on the appearance of turbulence on the colour duplex image (Figure 6.1, p.177), together with either an increase in blood velocity of more than 50%, or any decrease in velocity to below 40cm/sec. (Green et al, 1990).

Patients whose surgery had been carried out in Glasgow Royal Infirmary also underwent assessment as described above, colour duplex scans being performed by Dr. Alan Reid, Consultant Radiologist, using an ATL Ultramark 9 (HDI) scanner (Advanced Technology Laboratories, San Francisco, USA). In addition grafts were directly insonnated using a hand held 4 or 8Mhz probe attached to a Scimed PVL 50 portable vascular laboratory (Scimed, Bristol, UK). Pulse volume recordings and Doppler velocity tracings were obtained from the proximal and distal ends of the graft, using the Scimed PVL 50, and proximal and distal impedance measurements were calculated for the limb (Wyatt et al, 1991). These measurements were carried out by myself in conjunction with the research nurses in the Fraser Vascular Laboratory, Glasgow Royal Infirmary, and all patients in whom frequencies greater than 4Khz were detected on graft insonnation, or who had a duplex scan suggestive of graft stenosis, or an impedance value above 0.5, underwent intra-arterial digital subtraction angiography to determine whether or not there was evidence of graft stenosis or run-off deterioration.

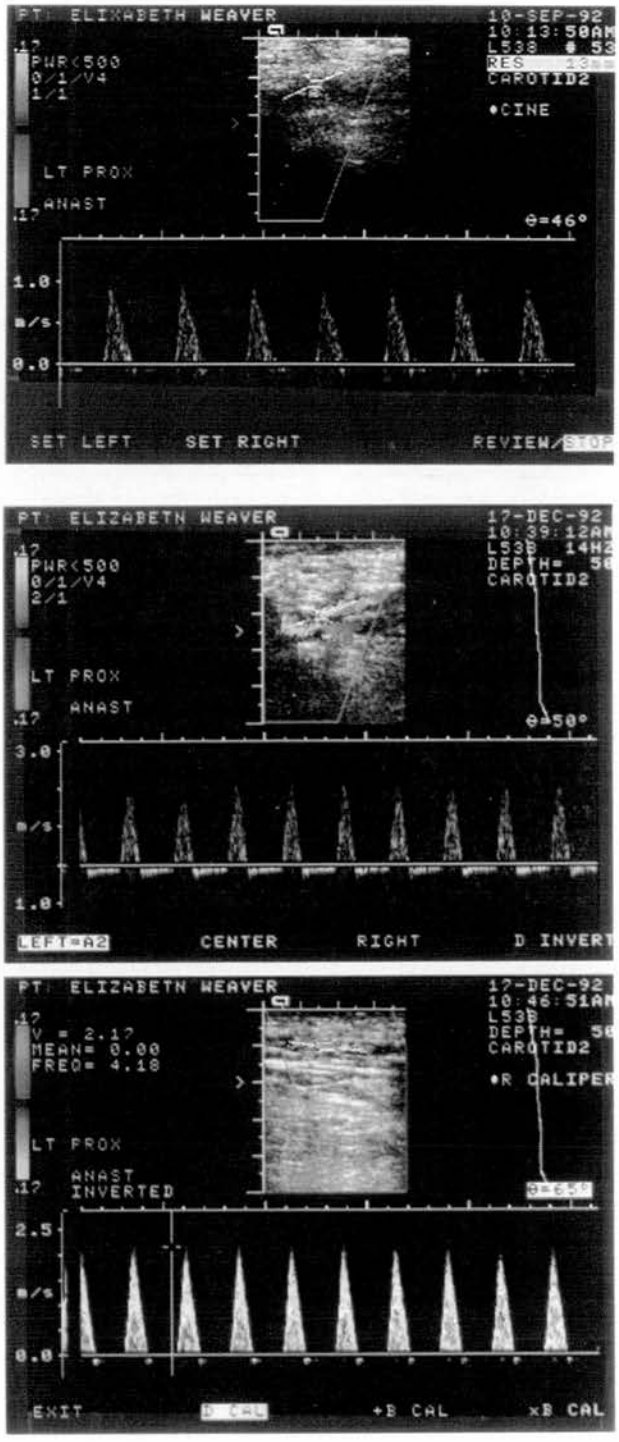


Figure 6.1: Duplex scans of above-knee femoropopliteal vein graft at 3 and 6 months post-insertion, there is evidence of graft stenosis in the later scan.

Measurement of the impedance value for a limb involves the computer assisted analysis of pulsatile pressure and flow signals: Measurement of Doppler flow velocity was made proximal to the graft origin using a 4 or 8 MHz Doppler probe, 12 to 15 velocity waveforms were obtained and the mean value stored in the PVL 50. A pneumatic cuff was then placed around the upper thigh, and linked to a pulse volume recorder contained within the PVL 50. A number of pulse volume waveforms were recorded and the mean stored in the PVL 50. An on-board computer was used to calculate an impedance score for the limb using Fourier transfer analysis of the 2 averaged waveforms (Wyatt et al, 1991). The process was repeated at the lower end of the graft (Figure 6.2, p.179). An impedance value of greater than 0.49 has previously been shown to be of value in detecting "at risk" grafts (Davies et al, 1993).

All patients in whom graft stenosis was proven on angiography were referred back to the consultant who had undertaken their initial surgery for consideration for further intervention to correct the stenosis.

Laboratory methods

Blood sampling was performed with minimum venous stasis, using a 19-gauge butterfly needle. Sample handling for all specimens was as described in chapter 2, citrated blood samples being transported to the laboratory in a vacuum flask at 4° Celsius, and the various assays carried out as described in Chapter 2. Smoking status was confirmed by clinical history and estimation of plasma carboxyhaemoglobin levels, as described in chapter 2. Carboxyhaemoglobin levels were measured prior to surgery and at each subsequent sampling time.

Statistical analyses

Univariate and multivariate analysis of pre-operative patient characteristics, blood rheology, and thrombotic mediators, were carried out by Dr Janet Love, Research Assistant, Robertson Centre for Biostatistics, University of Glasgow, in conjunction with Dr. G.D. Murray, Reader in Medical Statistics and Director, Robertson Centre for Biostatistics. Univariate analysis was performed by log rank testing (Peto et al, 1977) on an IBM-compatible microcomputer, using the BMDP statistical software package, module 1L, and multivariate analysis performed using Cox proportional hazards models (Module 2L) (BMDP Statistical Software Inc., Cork, Eire). For univariate analyses of continuous variables, patients were divided into 2 groups: those patients who had a value above the median value for the entire group, and those with a value below the median. In view of the highly skewed

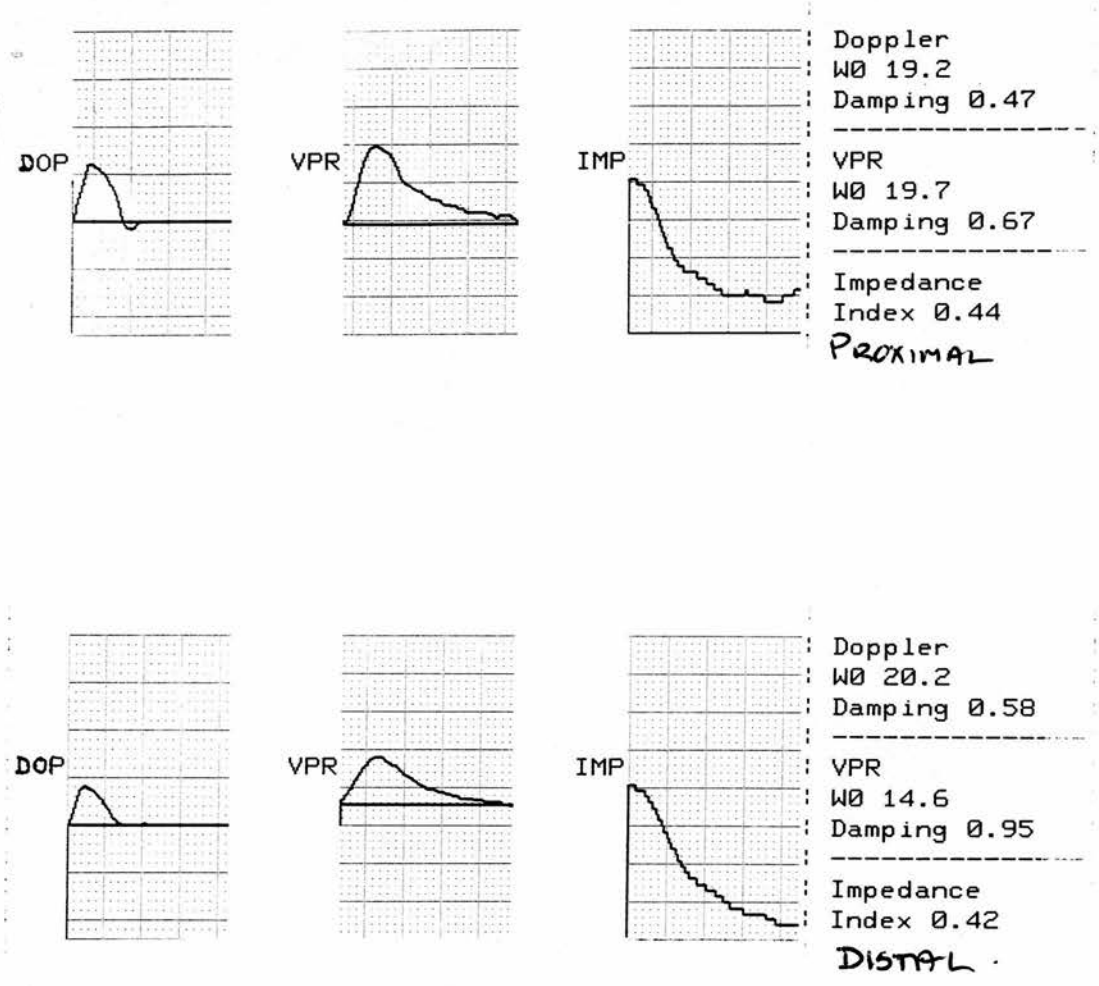


Figure 6.2: Impedance tracings obtained from a below knee femoropopliteal vein graft using the SciMed PVL 50 portable vascular laboratory.

distribution of values of FDP's, this variable was analysed after logarithmic transformation.

All other statistical analyses were carried out by myself with the CSS:Statistica package (Statsoft, Tulsa, USA), using non-parametric methods.

RESULTS

Graft and patient survival

There were 186 consecutive infra-inguinal bypass grafts performed on 165 patients. The mean age of patients at the time of surgery was 66 years (range 36-89 years), with a median of 67 years (interquartile range 60-72 yrs.). 15 patients underwent bilateral grafting during the period of the study, and a further 6 patients underwent redo bypass grafting after occlusion of a graft inserted during the study period. With the exception of 14 grafts, all operations were carried out as non-urgent procedures. Sixteen patients (10%) died with patent grafts during the period of the study (9 with vein grafts, 7 with synthetic), all but 3 deaths occurring within 3 months of operation, and 6 within 30 days of surgery. 8 patients (50%) died following a documented cardiac event, 2 from post-operative respiratory failure, 1 following massive pulmonary thromboembolism, and the cause of death was not stated in 5 patients. Forty-seven (47) grafts occluded within 1 year of surgery, giving a total of 63 grafts that were classed as having a poor outcome.

Almost half the grafts were performed in patients who had undergone prior vascular surgery, and in 70 cases (38%) surgery was carried out to relieve critical limb ischaemia (European Working Group on Critical Limb Ischaemia, 1990). Fifty percent of grafts were performed in current smokers (93 grafts), while 51 grafts (27%) were carried out in patients who had been non-smokers for at least 5 years (Table 6.1, p.181). Fifty percent (93) of the grafts were carried out in patients with evidence of other cardiovascular morbidity in addition to their peripheral vascular disease (angina, previous myocardial infarction, atrial fibrillation, transient ischaemic attacks or completed stroke). The severity of peripheral arterial disease in the study group, as determined by the Bollinger angiogram score (Chapter 3), was normally distributed (Figure 6.3, p.182). Patients with ischaemic ulceration and gangrene of digits were classified as having infected tissue, in addition to patients in whom cellulitis and infected ulceration was proven bacteriologically.

Characteristic	vein grafts (90)	synthetic grafts (96)	Chi^2 statistic	p value
Current smoker	55	38	8.61 (1)	p = 0.01
prior vascular surgery	41	51	1.04 (1)	n.s.
ischaemic heart disease	31	41	1.31 (1)	n.s.
critical limb ischaemia	35	35	0.11 (1)	n.s.
Male sex	60	60	0.25 (1)	n.s.
antiplatelet therapy	25	38	2.90 (1)	p = 0.10
limb sepsis/gangrene	26	26	0.06 (1)	n.s.
non-smoker (> 5 yrs)	21	30	1.48 (1)	n.s.
treated hypertension	18	27	1.69 (1)	n.s.
recent smoker	14	28	6.56 (1)	p = 0.05
cerebrovascular disease	10	17	1.66 (1)	n.s.
diabetes mellitus	11	15	0.36 (1)	n.s.
Atrial fibrillation	8	11	0.34 (1)	n.s.
treated hyperlipidaemia	4	5	0.07 (1)	n.s.
warfarin therapy	3	4	0.10 (1)	n.s.

Table 6.1: Details of patient characteristics in 186 consecutive infra-inguinal bypass grafts.

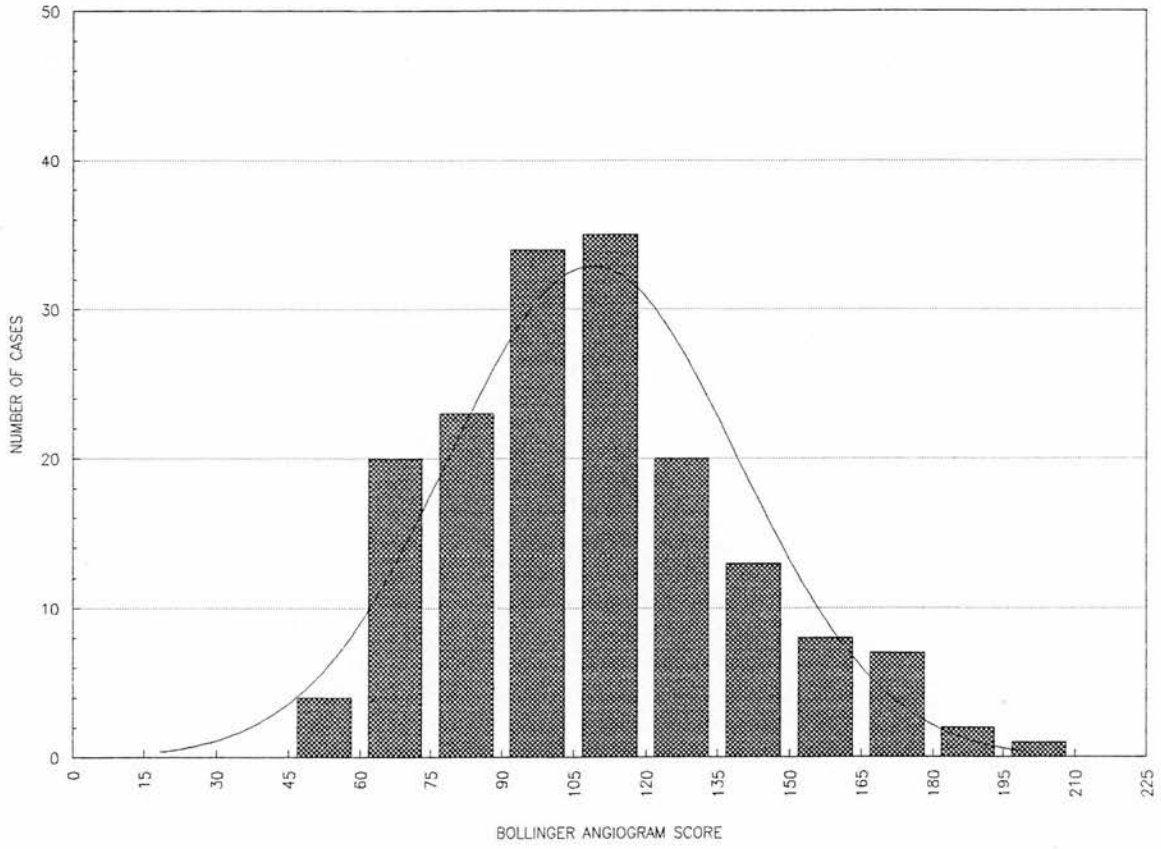


Figure 6.3: Distribution of Bollinger angiogram scores in patients undergoing infra-inguinal bypass grafting.

Synthetic grafts were removed from 2 patients while still patent, both as a consequence of graft infection, and these grafts have been excluded from subsequent analyses. There were therefore 184 grafts that made up the study group: 90 vein grafts and 94 synthetic grafts. Synthetic grafts consisted of 6mm. diameter PTFE (Gore-tex, W.L. Gore & Associates (UK) Ltd, Woking, Surrey. Impra-flex, Impra (UK) Ltd, Ascot, Berks.), or 8mm. gelatin coated knitted dacron grafts (Gelsoft, Vascutek Ltd, Inchinnan, Renfrewshire, UK.). A number of synthetic grafts also had a vein patch (Taylor et al, 1992) or cuff (Tyrrell & Wolfe, 1991) interposed at the distal anastomosis.

With the exception of 2 patients unable to attend for follow up, all grafts have been followed up for a mean of 310 days, with all but 30 of the grafts followed up for a full year. Patency of the 2 grafts lost to follow up has been confirmed by the patient's own General Practitioner. Table 6.2 (p.184) gives details of the types of graft used and the site of distal anastomosis, while the cumulative patency rates for vein and synthetic grafts in the 1st post-operative year are shown in figure 6.4 (p.185). Patient characteristics were similar in both vein and synthetic groups, with the exception of smoking habit, which was greater in patients undergoing vein grafting (Table 6.1).

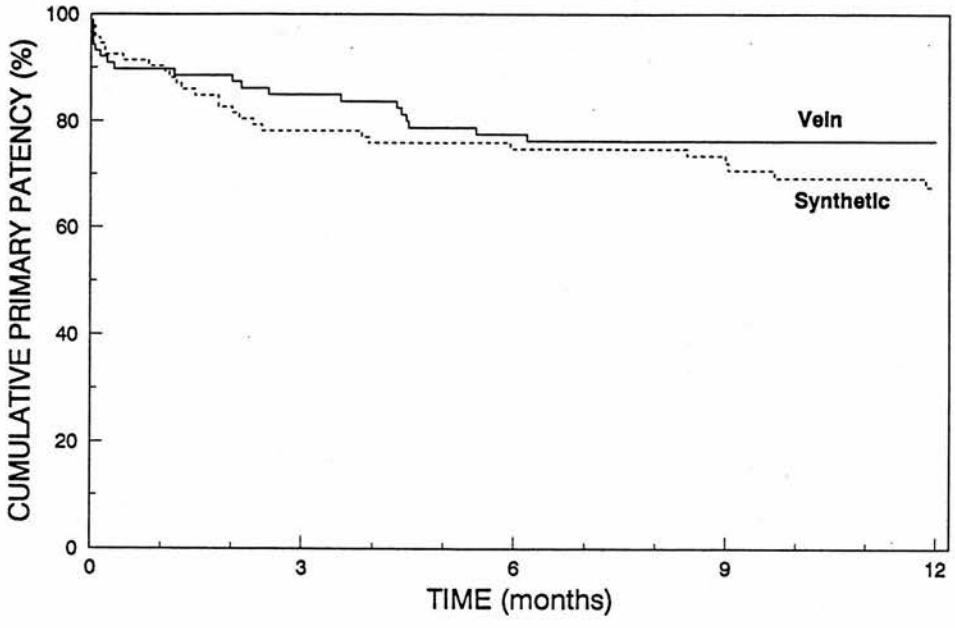
Patient characteristics and outcome

On univariate log rank testing a number of terms were found to be related to the time of primary endpoint, defined as death or graft occlusion within 1 year of surgery (Table 6.3, p.186). Female sex (Fig. 6.5, p.187), age over 67 years (Fig. 6.6, p.188), presence of limb sepsis or tissue necrosis (Fig. 6.7, p.188), emergency revascularisation (Fig. 6.8, p.190), single vessel run off (Fig. 6.9, p.191), a graft to distal popliteal or calf vessels (Fig. 6.10, p.192), a systolic ankle pressure of less than 71mm Hg (Fig 6.11, p.193), and an angiogram severity score (as described in chapter 3) of over 107 (Fig. 6.12, p.194) were all significantly related to death and graft occlusion within 1 year of surgery. There was no association between a poor outcome and graft material or current smoking habit, although the post-operative use of antiplatelet therapy (aspirin or dipyridamole), showed a trend towards a significant association with increased cumulative primary patency (Table 6.3, p.186, Fig. 6.13, p.195).

Subsequent multivariate analysis of the patient characteristics, carried out on the 130 cases in whom all data was complete, indicated that a low systolic ankle pressure (Chi^2 statistic 16.64, $p < 0.0001$), presence of limb sepsis or tissue necrosis (Chi^2 7.80, $p < 0.01$), prior vascular surgery (Chi^2 5.11, $p = 0.02$),

ANASTOMOSIS	PATENT	OCCLUDED (%)	TOTAL
Proximal popliteal			
reversed vein	46 (5)	8 (15%)	54
in-situ vein	--	----	--
PTFE	37 (3)	11 (23%)	48
PTFE & vein cuff	3	---	3
Dacron	8	2 (20%)	10
Distal popliteal			
Reversed vein	11 (2)	----	11
in-situ vein	3 (1)	3 (50%)	6
PTFE	6 (1)	5 (45%)	11
PTFE & vein cuff	4	----	4
Dacron	1	----	1
Crural vessel			
Reversed vein	1	1 (50%)	2
in-situ vein	9 (1)	8 (47%)	17
PTFE	4 (1)	6 (60%)	10
PTFE & vein cuff	4 (2)	3 (43%)	7
Dacron	--	----	--

Table 6.2: Outcome by site of distal anastomosis and material used in 184 infra-inguinal bypass grafts. Based on a mean follow-up period of 310 days. Figures in brackets in 'patent' column indicate number of deaths within period of follow-up.

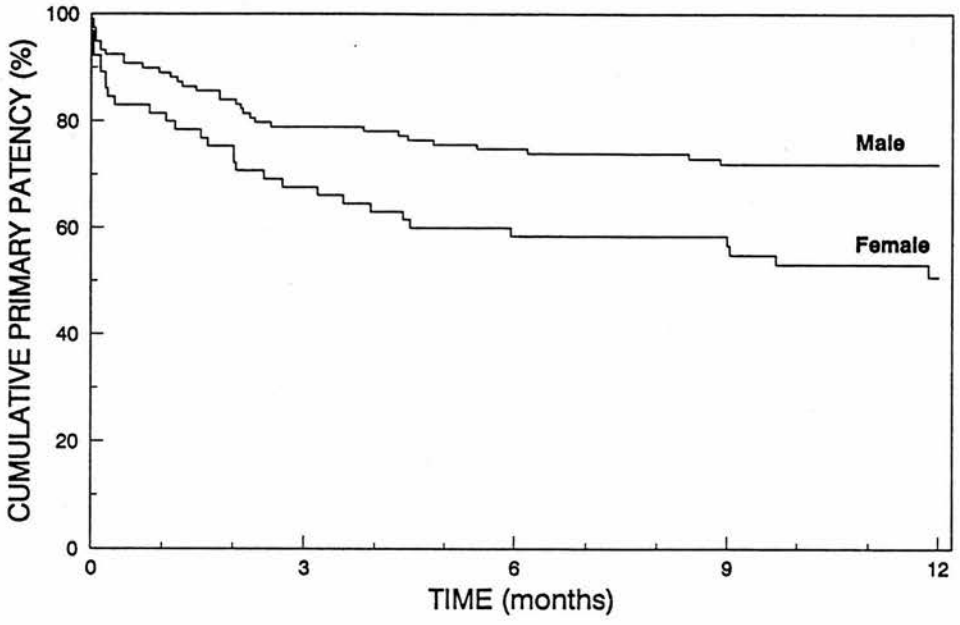


NUMBERS AT RISK					
Vein	90	70	62	50	40
Synthetic	94	68	65	54	40

Figure 6.4: 1 year cumulative patency curves in 90 vein and 94 synthetic infra-inguinal bypass grafts.

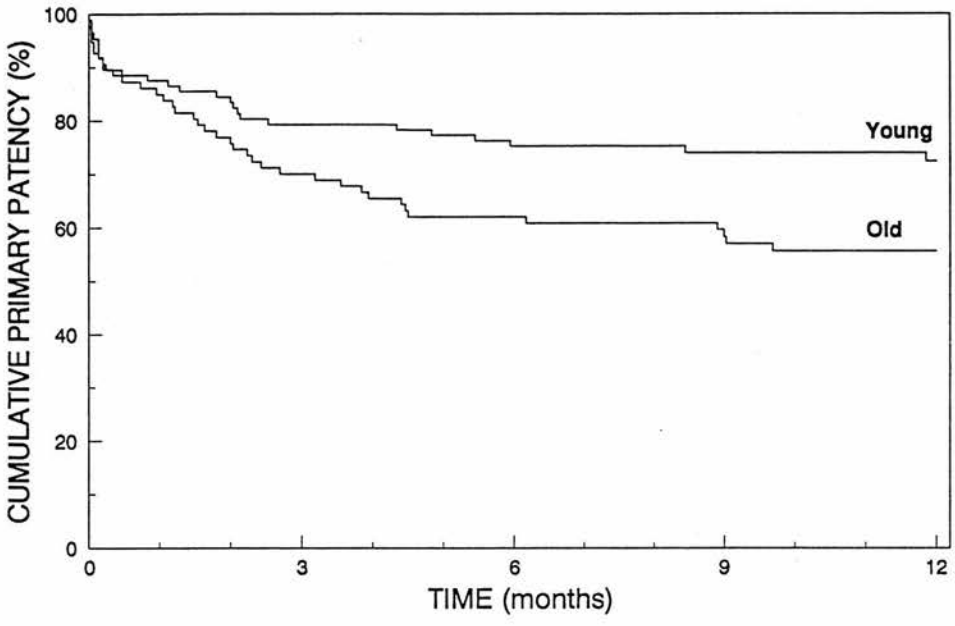
TERM	N	LOG RANK STATISTIC	REFER TO	p VALUE
No. calf vessels (0-3)	184	38.3	Chi^2 (3)	$p < 0.0001$
Infected limb (Y/N)	184	28.79	Chi^2 (1)	$p < 0.0001$
Anastomosis (AK/BK/calf)	184	25.54	Chi^2 (2)	$p < 0.0001$
Systolic ankle pressure	136	23.77	Chi^2 (1)	$P < 0.0001$
Elective (Y/N)	184	9.20	Chi^2 (1)	$p < 0.01$
Sex	184	7.20	Chi^2 (1)	$p < 0.01$
Bollinger score	173	5.92	Chi^2 (1)	$p = 0.02$
Age (years)	184	5.01	Chi^2 (1)	$p = 0.03$
Post-op. aspirin	184	3.38	Chi^2 (1)	$p = 0.07$
Post-op warfarin	184	2.67	Chi^2 (1)	$p = 0.10$
Diabetes (Y/N)	184	2.35	Chi^2 (1)	$p = 0.13$
Pre-op. warfarin	184	1.92	Chi^2 (1)	$p = 0.17$
Smoking (current/ex or non)	184	1.14	Chi^2 (1)	$p = 0.28$
Pre-op. aspirin	184	1.00	Chi^2 (1)	$p = 0.32$
other cardiovascular (Y/N)	184	0.72	Chi^2 (1)	$p = 0.40$
Hyperlipidaemia (Y/N)	184	0.61	Chi^2 (1)	$p = 0.43$
Graft (vein/synthetic)	184	0.31	Chi^2 (1)	$p = 0.58$
Prior vascular ops. (Y/N)	184	0.21	Chi^2 (1)	$p = 0.65$
Hypertension (Y/N)	184	0.01	Chi^2 (1)	$p = 0.90$

Table 6.3: Results of univariate survival analyses of patient characteristics in 184 grafts. Primary endpoint defined as death or graft occlusion. Significance of variables with continuous values are based on median split (see text).



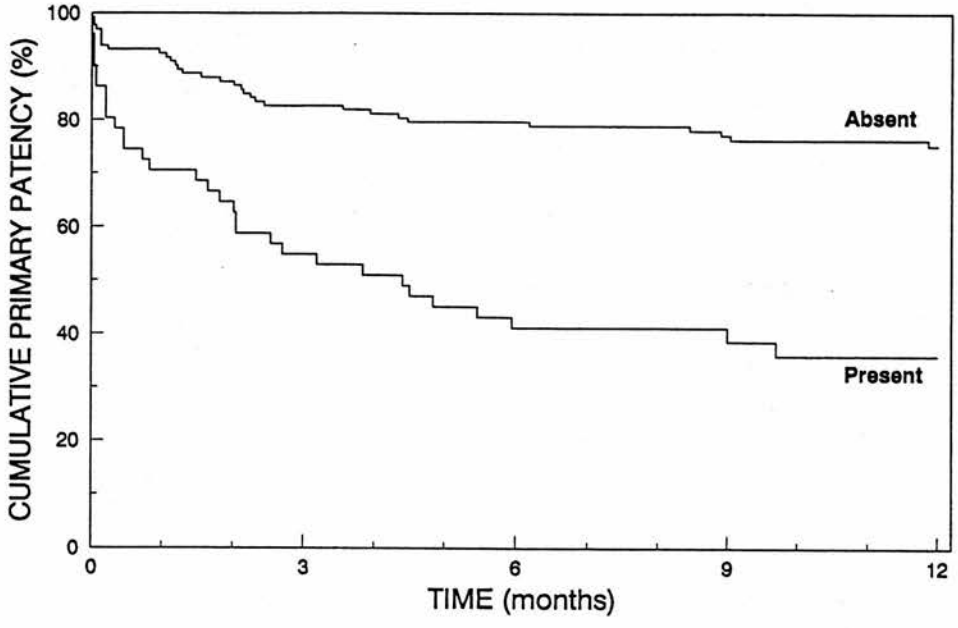
NUMBERS AT RISK					
Male	119	94	89	71	58
Female	65	44	38	33	22

Figure 6.5: Cumulative graft and patient survival by sex, based on univariate log rank analysis. 66 grafts in women, 120 grafts in men.



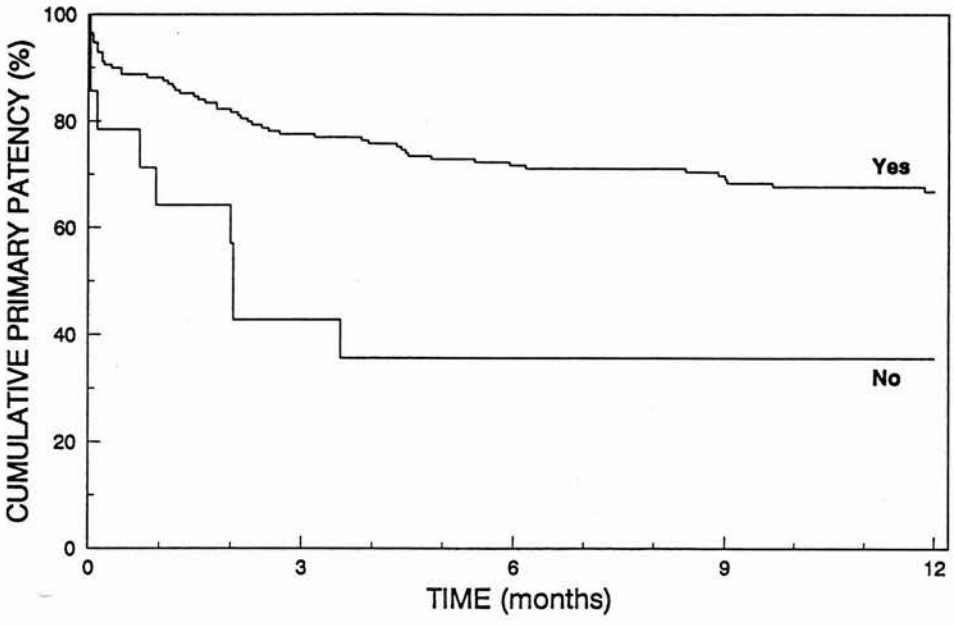
NUMBERS AT RISK					
Young	97	77	73	58	45
Old	87	61	54	46	35

Figure 6.6: Cumulative graft and patient survival by age, based on univariate log rank analysis. "Young" refers to cases with an age below the median value of 67 years, and "old" to those with an age above this.



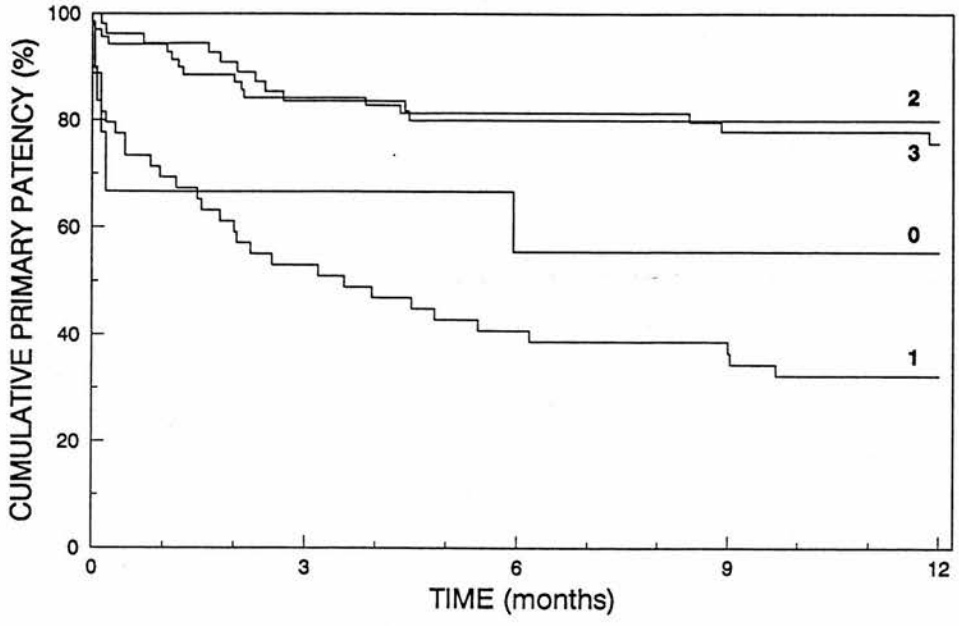
NUMBERS AT RISK					
Absent	133	110	106	88	69
Present	51	28	21	16	11

Figure 6.7: Cumulative graft and patient survival for patients with and without limb sepsis, based on univariate log rank analysis.



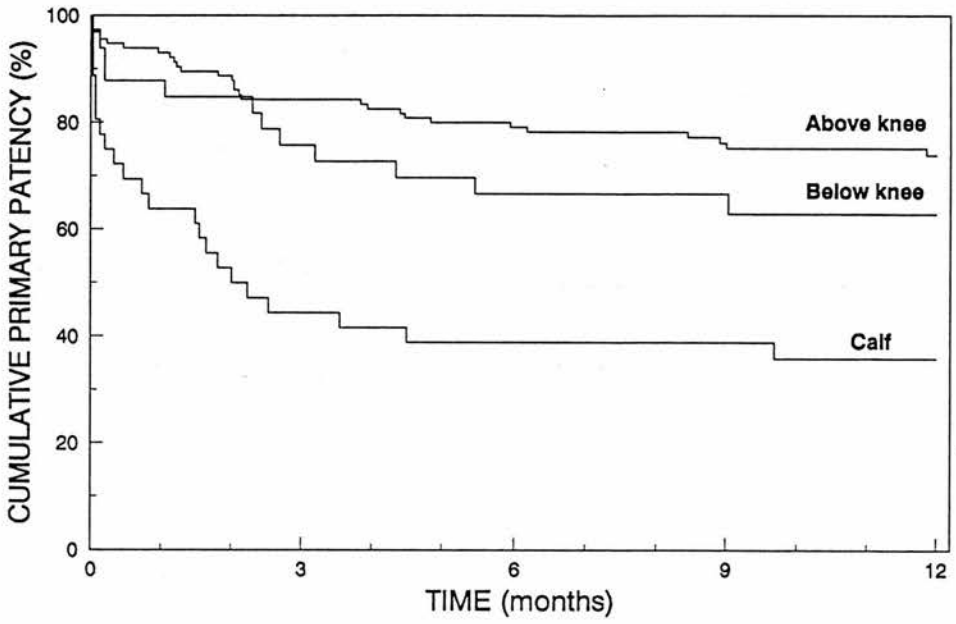
NUMBERS AT RISK					
No	14	6	5	3	3
Yes	170	132	122	101	77

Figure 6.8: Cumulative graft and patient survival as determined by whether or not surgery was carried out as an elective procedure, based on univariate log rank analysis.



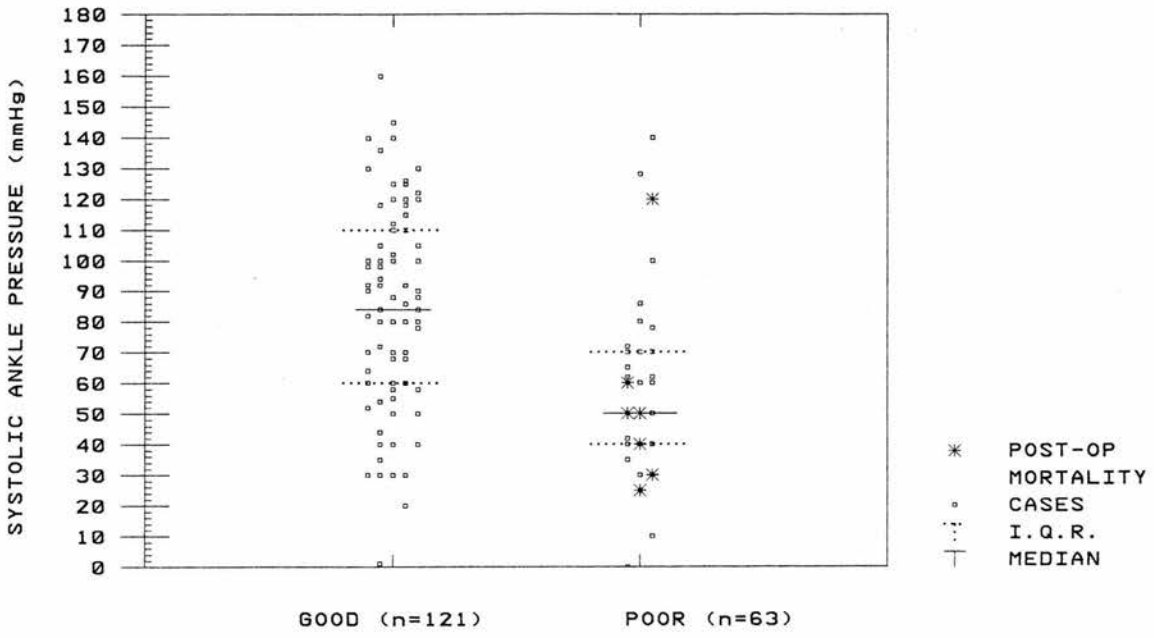
NUMBERS AT RISK					
0	9	6	5	5	2
1	49	26	20	18	11
2	55	46	44	37	31
3	70	59	57	44	36

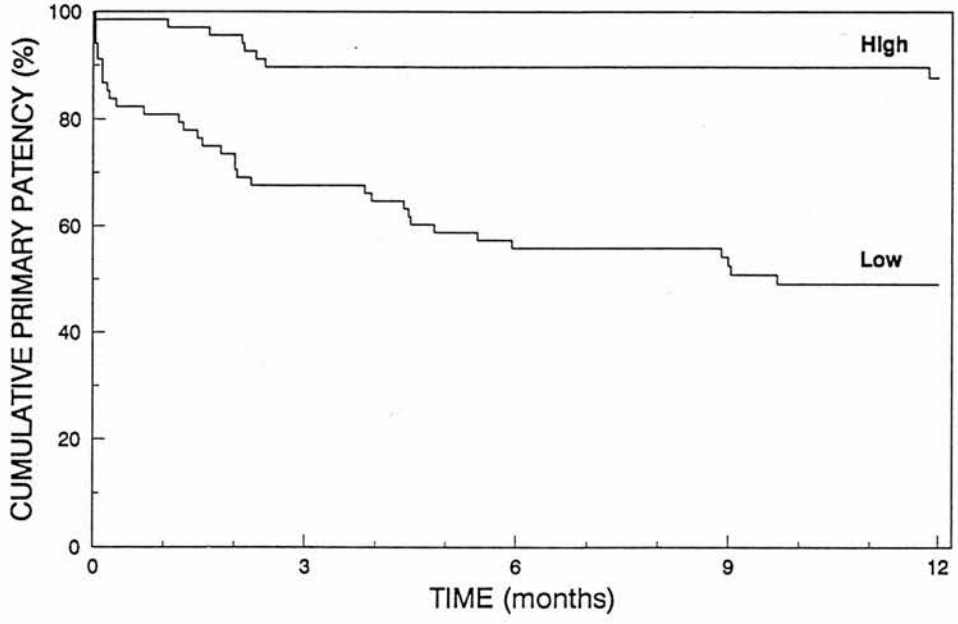
Figure 6.9: Cumulative graft and patient survival by number of run-off vessels (0-3 calf vessels), based on univariate log rank analysis.



NUMBERS AT RISK					
Above knee	115	97	91	73	59
Below knee	33	25	22	18	14
Calf	36	16	14	13	7

Figure 6.10: Cumulative graft and patient survival by site of distal anastomosis, based on univariate log rank analysis.

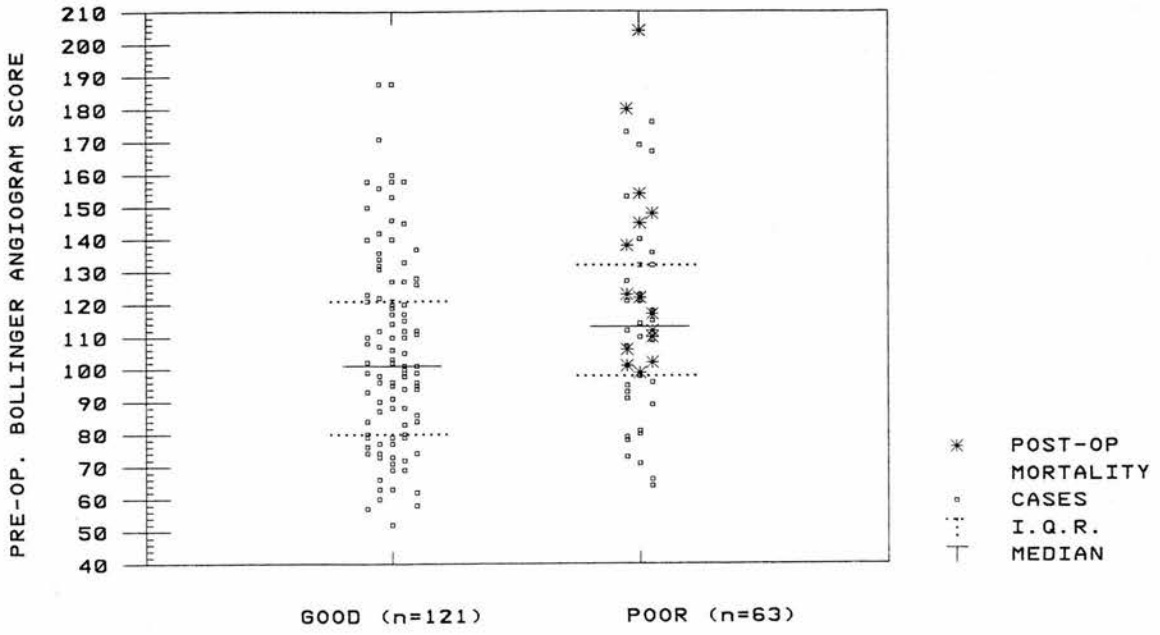


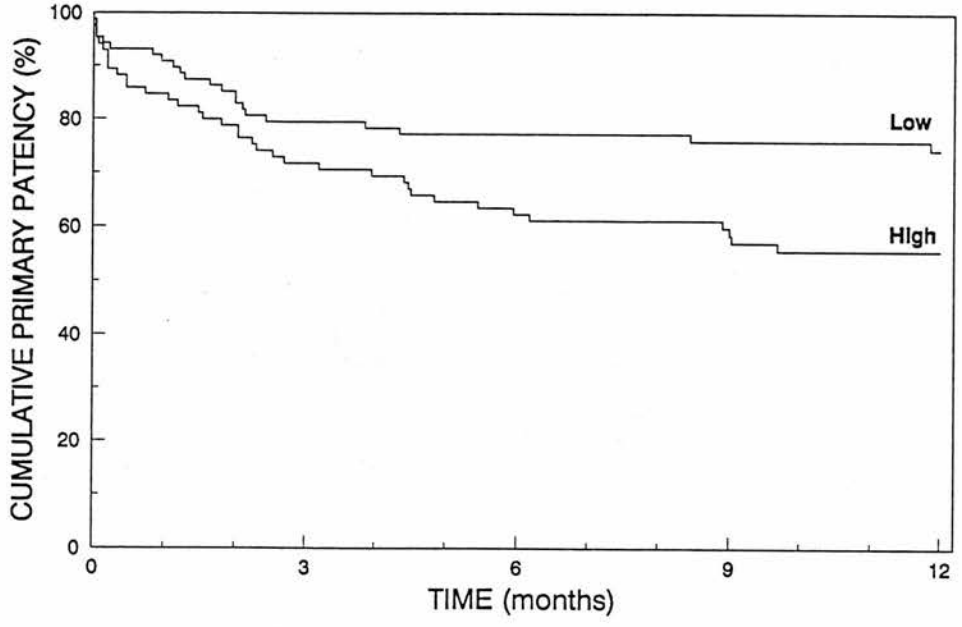


NUMBERS AT RISK

Low	68	46	38	32	23
High	68	61	61	53	44

Figure 6.11: Pre-operative systolic ankle pressure values by outcome, together with cumulative graft and patient survival. "High" refers to cases with a pre-operative systolic ankle pressure above the median value of 71mm Hg, and "low" to cases with a value below this..

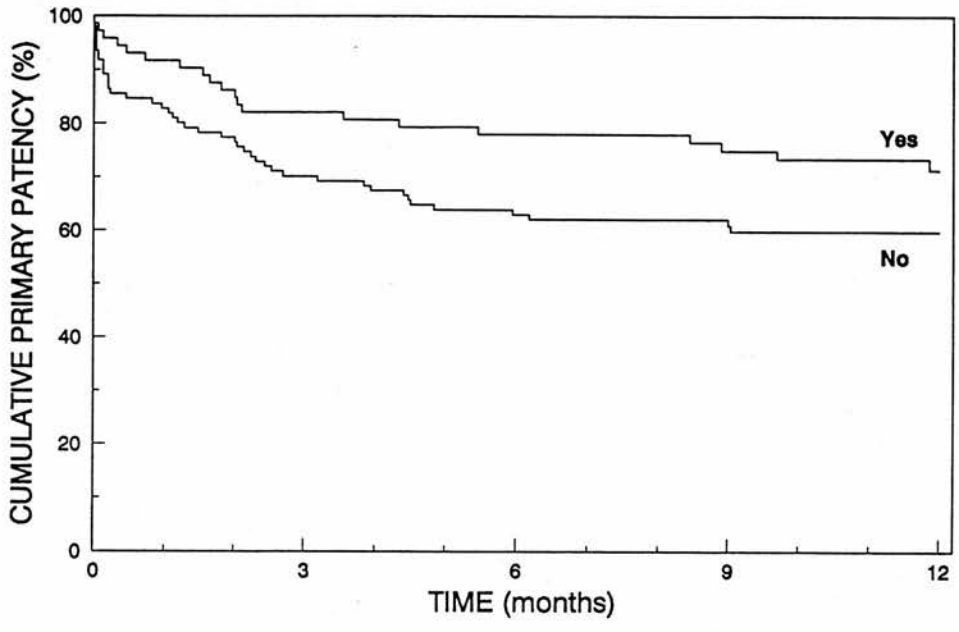




NUMBERS AT RISK

Low	88	70	68	55	46
High	85	61	53	43	29

Figure 6.12: Pre-operative Bollinger angiogram scores by outcome, together with cumulative graft and patient survival. "High" refers to cases with a Bollinger score above the median value of 107, "low" to patients with a value below this level.



NUMBERS AT RISK					
No	111	78	70	55	45
Yes	73	60	57	49	35

Figure 6.13: Cumulative graft and patient survival by antiplatelet therapy, based on univariate log rank analysis.

female sex (Chi^2 4.93, $p = 0.03$), and emergency infra-inguinal bypass grafting (Chi^2 4.78, $p = 0.03$), were all independently predictive of subsequent death and graft occlusion in the 1 year following revascularisation surgery.

Blood rheology and outcome

Rheological parameters were not significantly related to graft occlusion or death on univariate analysis (Table 6.4, p.197), with the exception of lower red cell aggregation (Myrenne) which showed a trend towards a significant association with poor outcome following surgery. Both reduced haematocrit (Fig. 6.14, p.198), and haemoglobin (Fig. 6.15, p.199) were related to graft occlusion and death on univariate analysis, as were elevated platelet (Fig 6.16, p.200) and white cell counts (Fig. 6.17, p.201).

Multivariate analysis indicated that an elevated platelet count was the only haemorrheological variable that improved on a predictive model based solely on patient background details (Table 6.5, p.202).

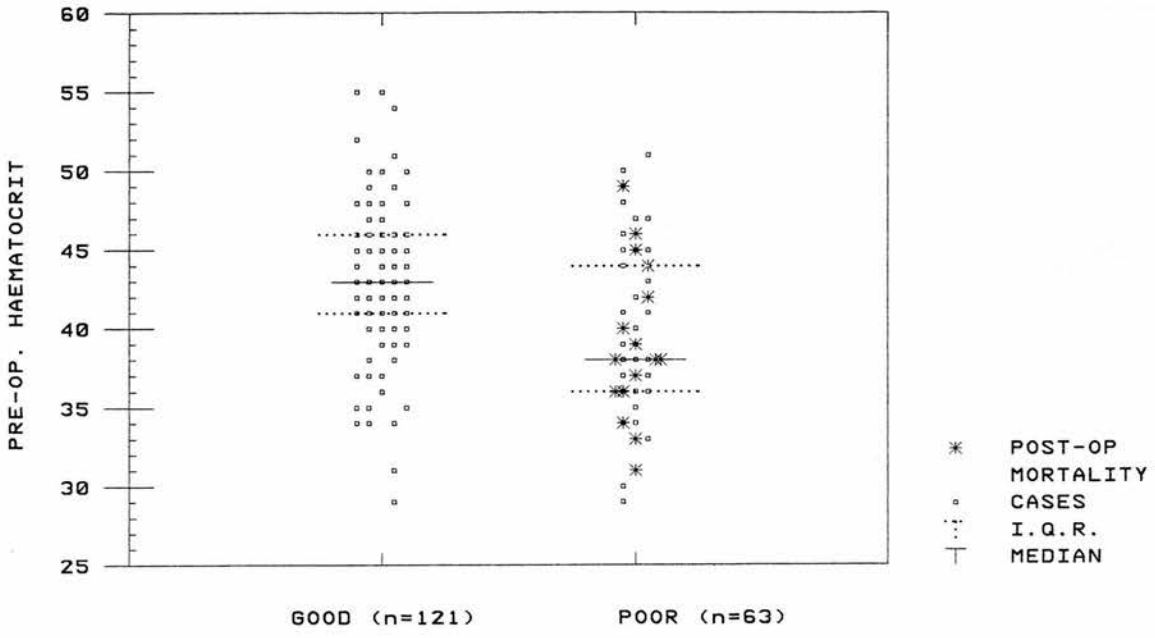
Thrombotic mediators and outcome

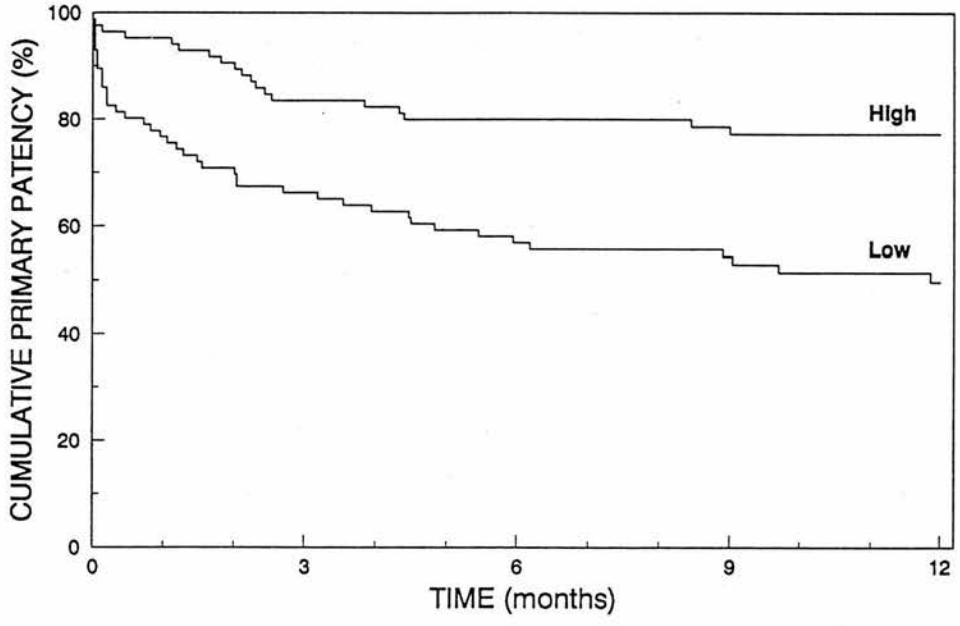
Increased pre-operative plasma fibrinogen (Fig. 6.18, p.203), increased von Willebrand Factor (Fig. 6.19, p.204), increased fibrin turnover (Cross-linked FDP's (log(FDP)), Fig. 6.20, p.205), and decreased factor VII activity (Fig. 6.21), were all related to post-operative graft occlusion and patient death on univariate analysis while pre-operative t.P.A. and P.A.I. activity (Table 6.4) were unrelated to the subsequent post-operative course.

On multivariate analysis, only elevated pre-operative plasma von Willebrand Factor was independently predictive of subsequent graft occlusion and patient death when tested within a model based on patient background information (Table 6.5). When this was then added to the model it was found that prior vascular surgery, and emergency revascularisation surgery were no longer significant terms in the model, and pre-operative plasma von Willebrand Factor level (Chi^2 statistic 14.14, $p < 0.001$), systolic ankle pressure (Chi^2 8.30, $p < 0.01$), presence of infection or tissue necrosis (Chi^2 6.33, $p = 0.01$), and female sex (Chi^2 5.01, $p = 0.03$), were the only terms independently related to graft and patient survival. Tables 6.6 and 6.7 summarise the results of testing all other variables studied, within this final model (p.207-208).

TERM	N	LOG RANK STATISTIC	REFER TO	p VALUE
vWF	184	27.45	Chi^2 (1)	$p < 0.0001$
Haemoglobin	184	17.86	Chi^2 (1)	$p < 0.0001$
Platelets	184	13.81	Chi^2 (1)	$p < 0.001$
Haematocrit	171	13.78	Chi^2 (1)	$p < 0.001$
Fibrinogen	183	12.37	Chi^2 (1)	$p < 0.001$
Log(FDP)	182	11.96	Chi^2 (1)	$p < 0.001$
Factor VII	178	9.30	Chi^2 (1)	$p < 0.01$
Cholesterol	175	6.16	Chi^2 (1)	$p = 0.01$
W.C.C.	184	3.94	Chi^2 (1)	$p < 0.05$
Red cell aggregation	166	3.37	Chi^2 (1)	$p = 0.07$
Plasma viscosity	172	2.11	Chi^2 (1)	$p = 0.15$
Relative blood viscosity	156	1.27	Chi^2 (1)	$p = 0.26$
t.P.A.	164	0.97	Chi^2 (1)	$p = 0.32$
P.A.I.	168	0.65	Chi^2 (1)	$p = 0.42$
Blood viscosity	156	0.23	Chi^2 (1)	$p = 0.63$

Table 6.4: Associations between pre-operative rheology and thrombotic mediators, and poor outcome (death or graft occlusion) following infra-inguinal bypass grafting (184 grafts). Univariate analysis with statistical significance based on logrank testing.

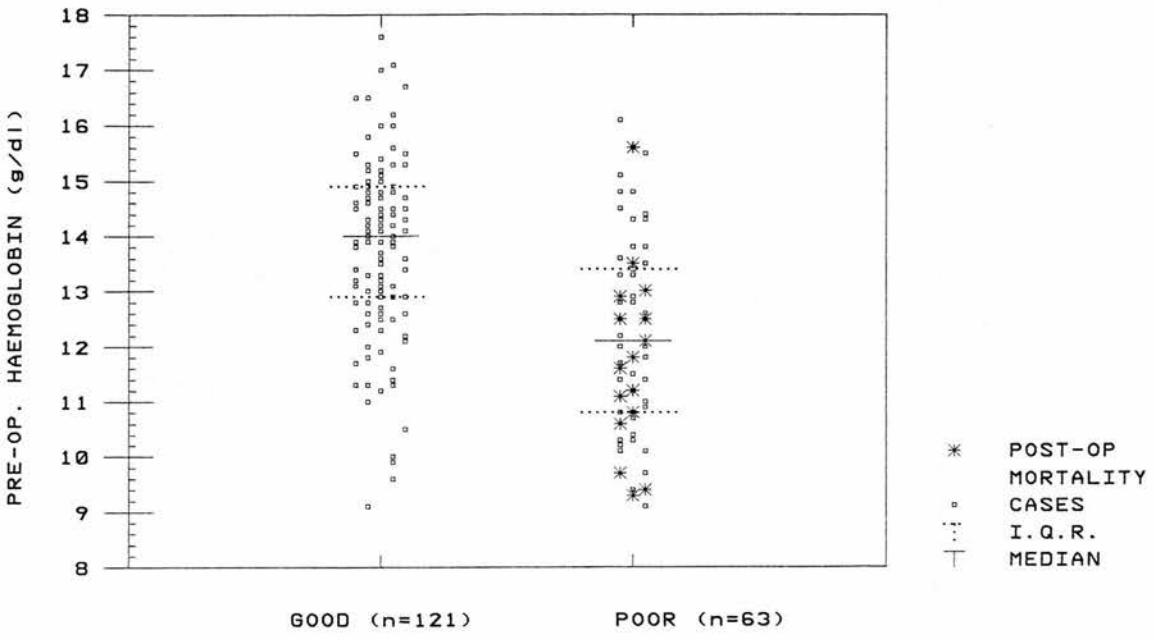


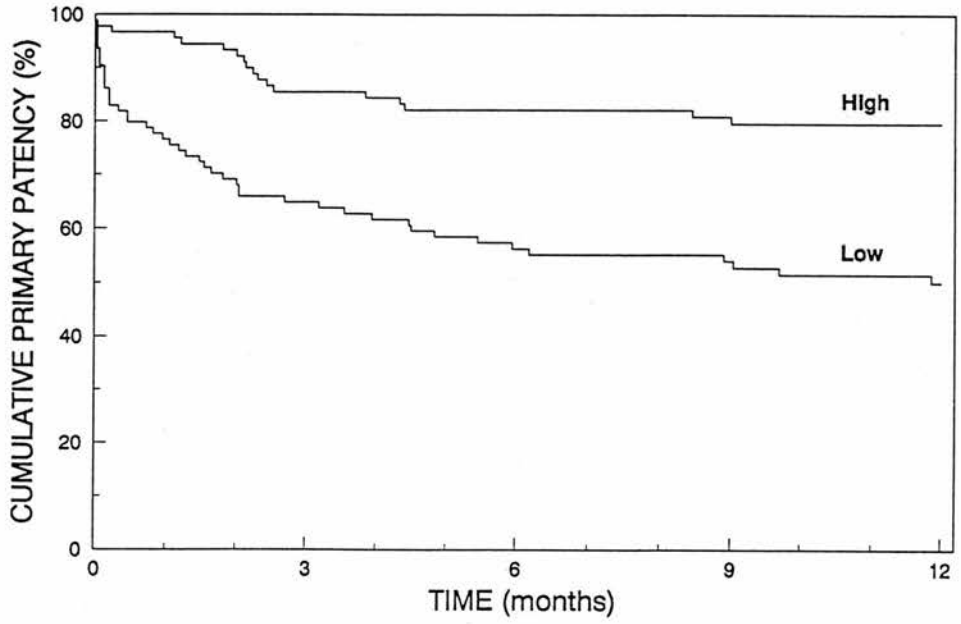


NUMBERS AT RISK

Low	86	57	49	38	28
High	85	71	68	56	47

Figure 6.14: Pre-operative haematocrit values by outcome, together with cumulative graft and patient survival. High values are cases with a pre-operative haematocrit above the median value of 42, low have values below this level.

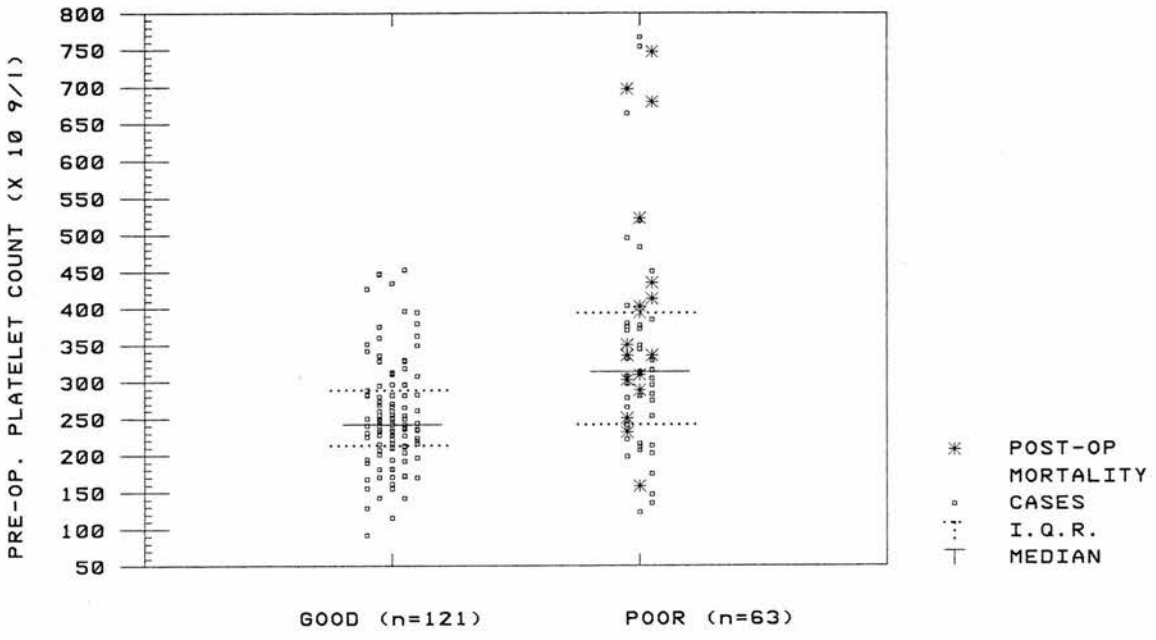


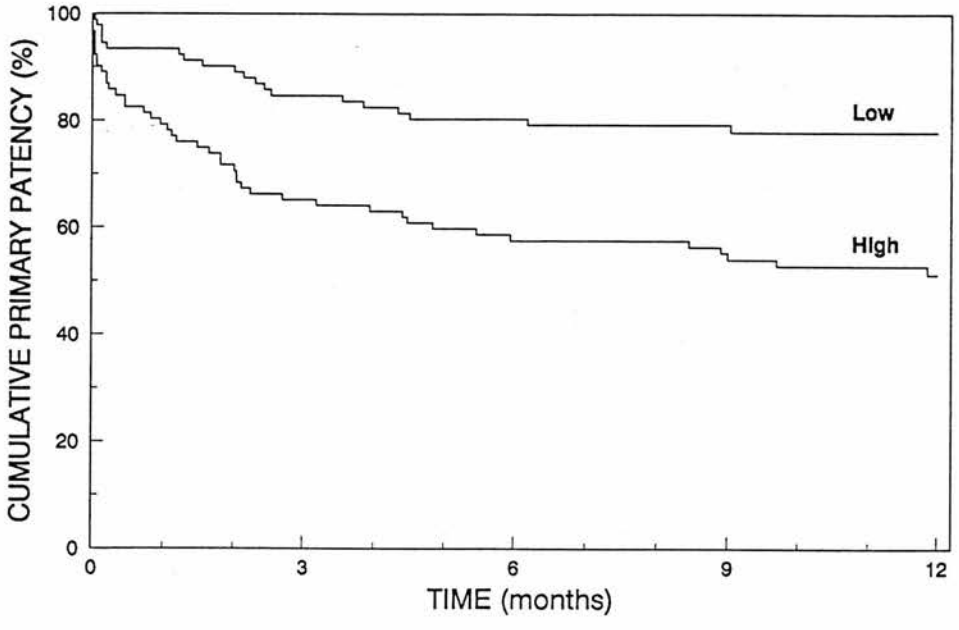


NUMBERS AT RISK

Low	94	61	53	44	34
High	90	77	74	60	46

Figure 6.15: Pre-operative haemoglobin by outcome, together with cumulative graft and patient survival. High values are above the median pre-operative value of 13.4g/dl, low have values below this.

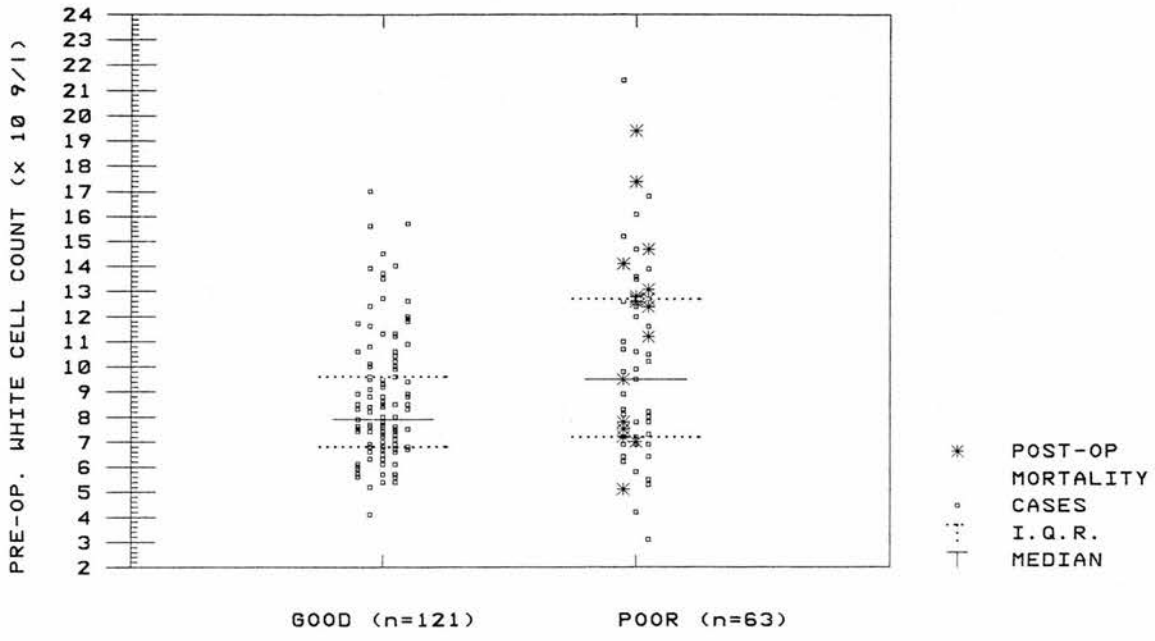


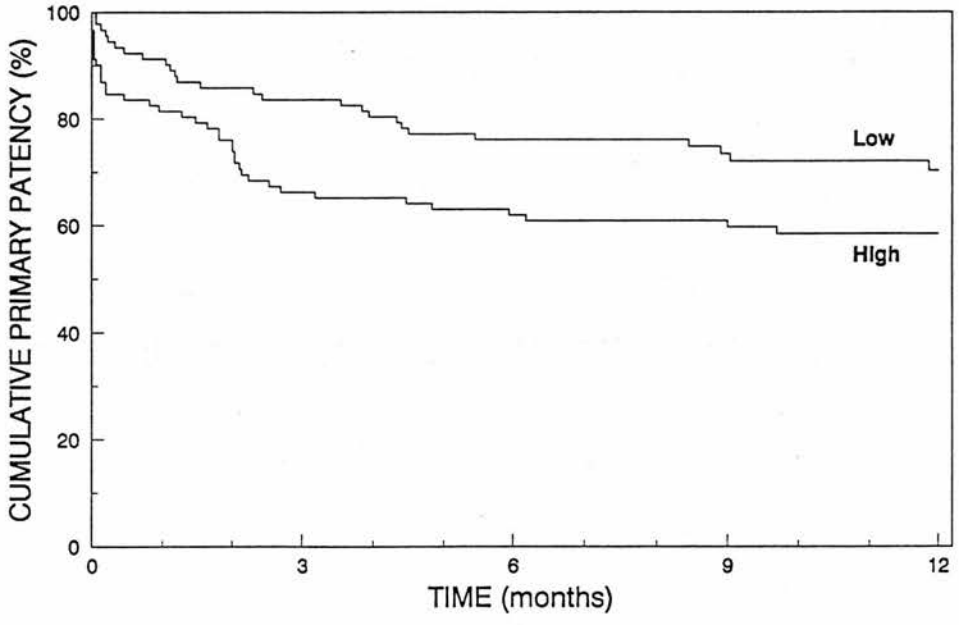


NUMBERS AT RISK

Low	92	78	74	57	47
High	92	60	53	47	33

Figure 6.16: Pre-operative platelet count by outcome, together with cumulative graft and patient survival. High values are above the median pre-operative platelet count of 258 x 10^9/l. Low are values below this.





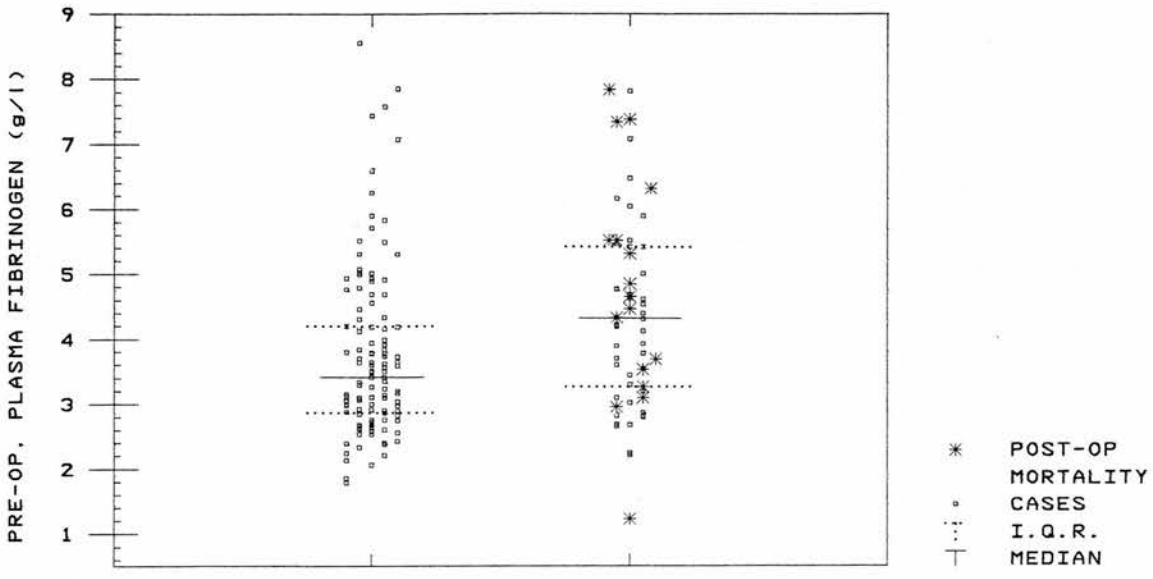
NUMBERS AT RISK

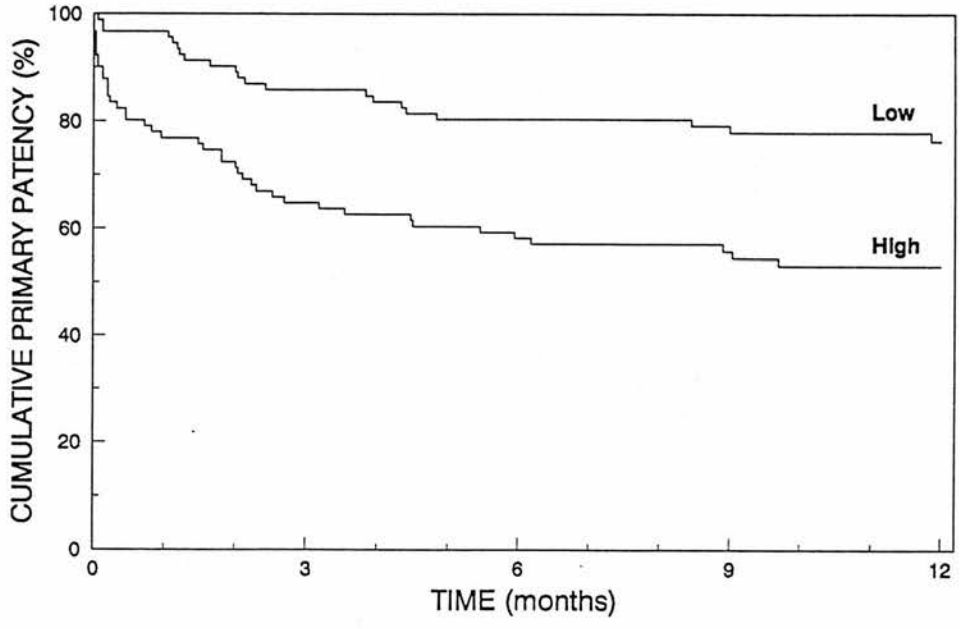
Low	92	77	70	53	41
High	92	61	57	51	39

Figure 6.17: Pre-operative white cell count by outcome, together with cumulative graft and patient survival. High values refer to pre-operative white cell count above the median value of 8.3 x 10^9/l. Low are cases with a white cell count below this level.

TERM	N	CHI^2 STATISTIC	REFER TO	p VALUE
vWF	130	7.88	Chi^2 (1)	$p < 0.01$
Platelets	130	4.09	Chi^2 (1)	$p = 0.04$
t.P.A.	118	3.22	Chi^2 (1)	$p = 0.07$
Hct.	120	2.19	Chi^2(1)	$p = 0.14$
Factor VII	126	1.48	Chi^2 (1)	$p = 0.22$
Red cell aggregation	119	1.25	Chi^2 (1)	$p = 0.26$
Relative blood viscosity	114	0.56	Chi^2 (1)	$p = 0.45$
Cholesterol	125	0.56	Chi^2 (1)	$p = 0.46$
W.C.C.	130	0.42	Chi^2 (1)	$p = 0.52$
Plasma viscosity	125	0.28	Chi^2 (1)	$p = 0.59$
Fibrinogen	129	0.06	Chi^2 (1)	$p = 0.81$
Log(FDP)	130	0.05	Chi^2 (1)	$p = 0.82$
P.A.I.	120	0.03	Chi^2 (1)	$p = 0.86$
Blood viscosity	114	0.01	Chi^2 (1)	$p = 0.92$
Haemoglobin	130	0.00	Chi^2 (1)	$p = 0.97$

Table 6.5: Associations between pre-operative rheology and thrombotic mediators, and poor outcome (death or graft occlusion) following infra-inguinal bypass grafting (184 grafts). Multivariate analysis by Cox's proportional hazards model, after adjusting for patient background information (sex, systolic ankle pressure, limb infection, prior vascular surgery, emergency revascularisation).

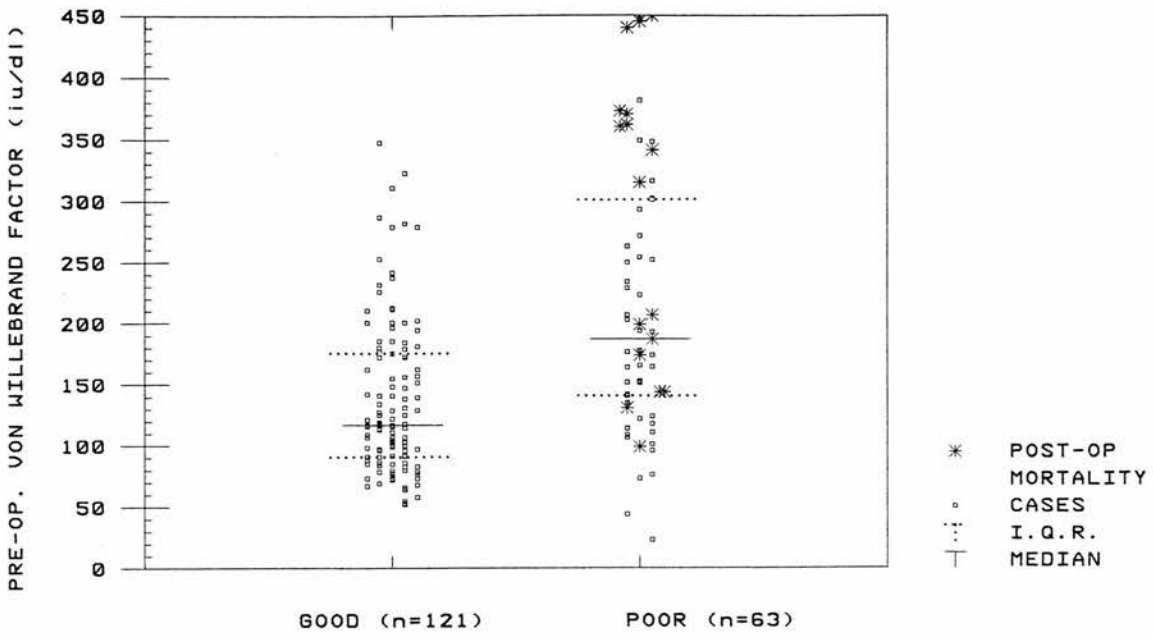


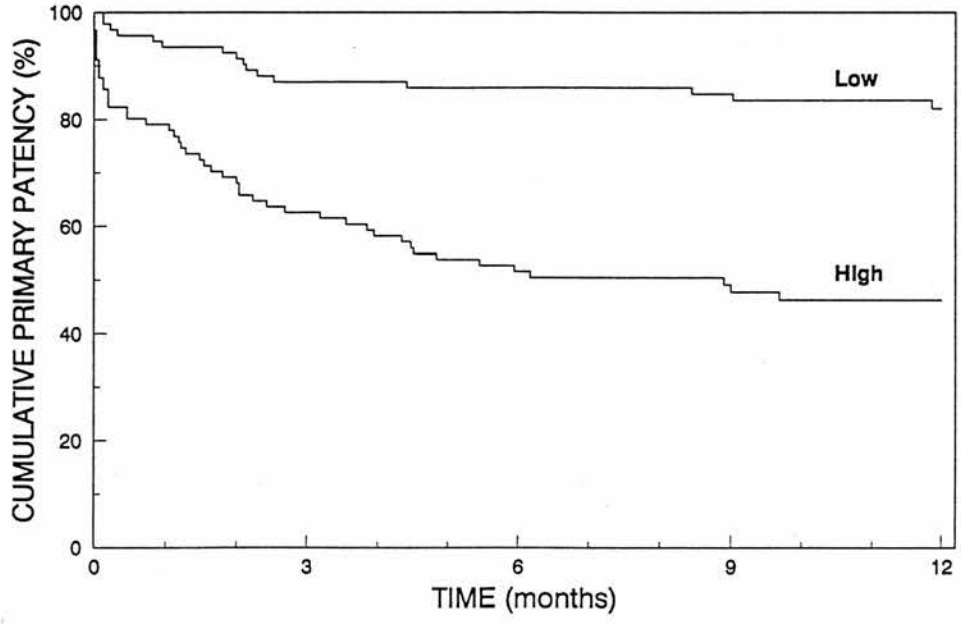


NUMBERS AT RISK

Low	92	79	74	63	50
High	91	59	53	41	30

Figure 6.18: Pre-operative plasma fibrinogen levels by outcome, together with cumulative graft and patient survival. High refers to pre-operative plasma fibrinogen levels above the median value of 3.6g/l, low to levels below this.

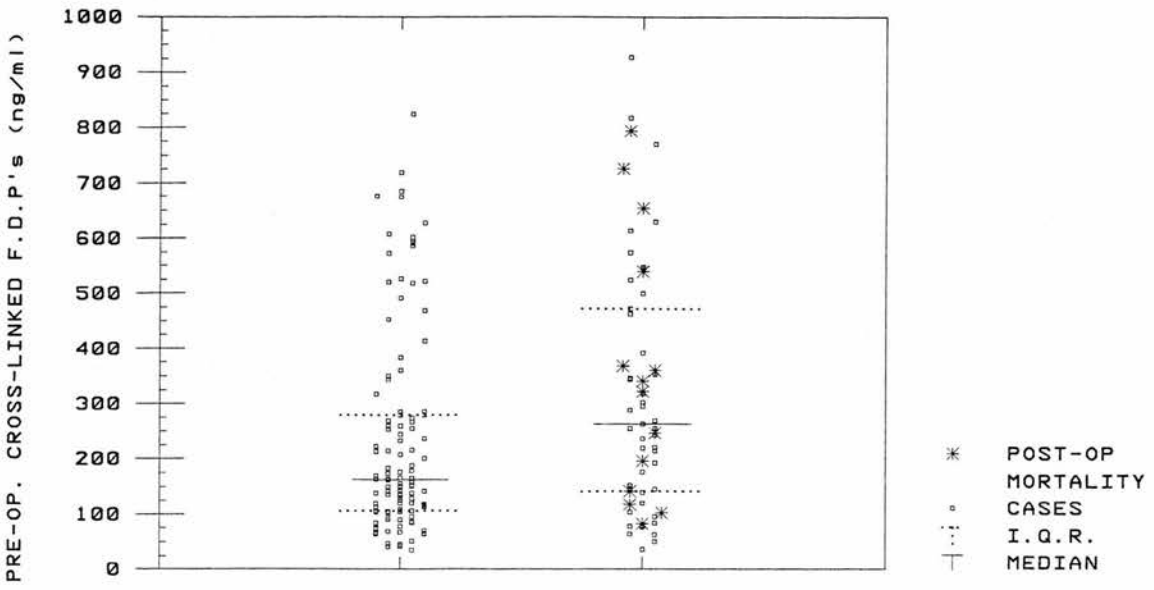


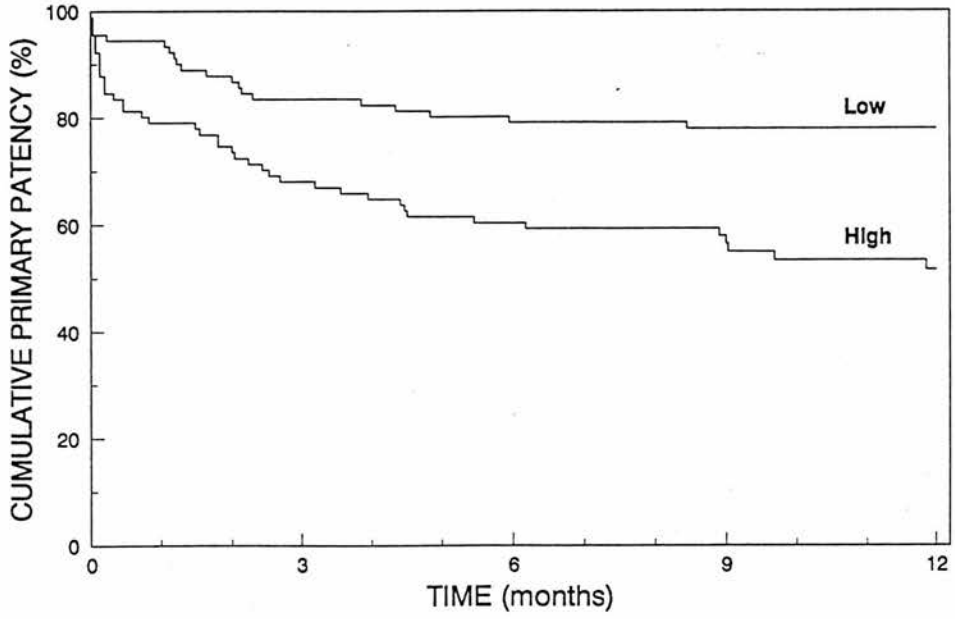


NUMBERS AT RISK

Low	93	81	80	70	55
High	91	57	47	34	25

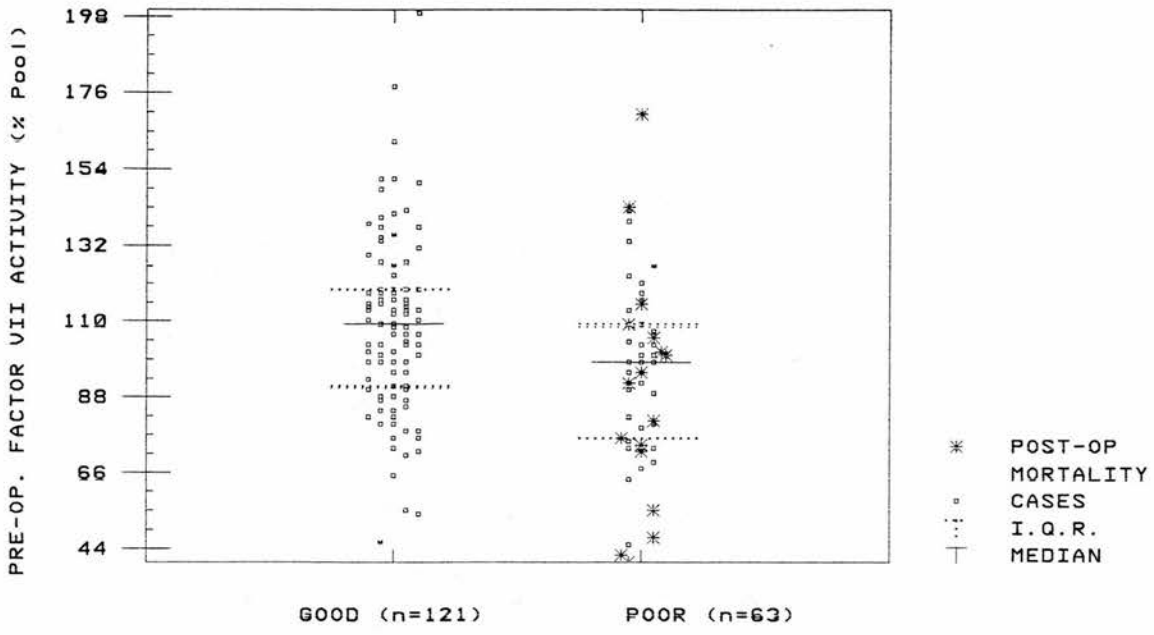
Figure 6.19: Pre-operative von Willebrand Factor levels by outcome, together with cumulative graft and patient survival. High values are those above the median pre-operative vWF level of 141iu/dl, low are values below this level.

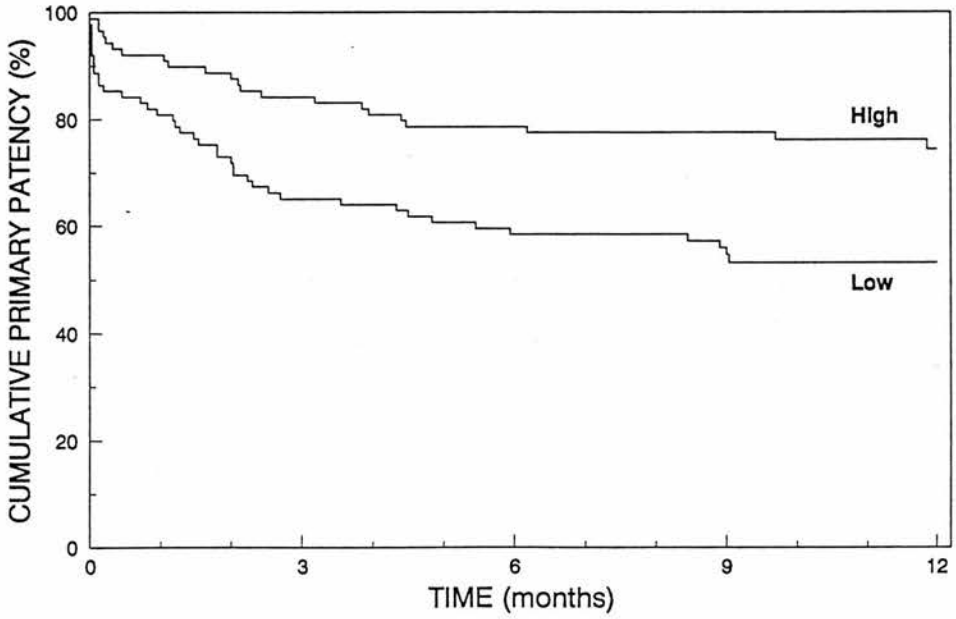




NUMBERS AT RISK					
Low	91	76	72	64	51
High	91	62	55	40	29

Figure 6.20: Pre-operative cross-linked FDP's by outcome, together with cumulative graft and patient survival by log(FDP). High values are those above the median pre-operative value for log(FDP) of 5.223, which is equivalent to an FDP level of 186 ng/ml. Low values are levels below this.





NUMBERS AT RISK

Low	89	58	52	43	34
High	89	75	70	57	42

Figure 6.21: Pre-operative Factor VII levels by outcome, together with cumulative graft and patient survival. High values refer to Factor VII levels above the pre-operative median level of 106%. Low refers to values below this.

TERM	N	CHI^2 STATISTIC	REFER TO	p VALUE
Diabetes (Y/N)	130	3.03	Chi^2 (1)	p = 0.08
Anastomosis (AK/BK/calf)	130	2.38	Chi^2 (2)	p = 0.30
Post-op warfarin	130	2.11	Chi^2 (1)	p = 0.15
Prior vascular ops. (Y/N)	130	1.81	Chi^2 (1)	p = 0.18
Pre-op. warfarin	130	1.57	Chi^2 (1)	p = 0.21
Post-op. aspirin	130	1.33	Chi^2 (1)	p = 0.25
Hyperlipidaemia (Y/N)	130	1.03	Chi^2 (1)	p = 0.31
Elective (Y/N)	130	0.68	Chi^2 (1)	p = 0.41
Bollinger score	130	0.20	Chi^2 (1)	p = 0.65
Pre-op. aspirin	130	0.18	Chi^2 (1)	p = 0.67
No. calf vessels (0-3)	130	0.06	Chi^2 (3)	p = 0.81
Hypertension (Y/N)	130	0.05	Chi^2 (1)	p = 0.83
Age	130	0.04	Chi^2 (1)	p = 0.84
Smoking (current/ex or non)	130	0.03	Chi^2 (1)	p = 0.87
other cardiovascular (Y/N)	130	0.00	Chi^2 (1)	p = 0.95
Graft (vein/synthetic)	130	0.00	Chi^2 (1)	p = 0.99

Table 6.6: Significance of patient characteristics on multivariate survival analysis after correcting for the effects of von Willebrand Factor, systolic ankle pressure, sex, and limb sepsis.

TERM	N	CHI^2 STATISTIC	REFER TO	p VALUE
Platelets	130	2.77	Chi^2 (1)	p = 0.10
Red cell aggregation	119	2.25	Chi^2 (1)	p = 0.13
t.P.A.	118	1.98	Chi^2 (1)	p = 0.16
Log(FDP)	130	1.66	Chi^2 (1)	p = 0.20
Haematocrit	120	0.63	Chi^2 (1)	p = 0.43
Plasma viscosity	125	0.56	Chi^2 (1)	p = 0.45
Relative blood viscosity	114	0.45	Chi^2 (1)	p = 0.50
Factor VII	126	0.40	Chi^2 (1)	p = 0.53
Blood viscosity	114	0.37	Chi^2 (1)	p = 0.54
Fibrinogen	129	0.22	Chi^2 (1)	p = 0.64
P.A.I.	120	0.13	Chi^2 (1)	p = 0.71
Cholesterol	125	0.07	Chi^2 (1)	p = 0.80
W.C.C.	130	0.03	Chi^2 (1)	p = 0.86
Haemoglobin	130	0.00	Chi^2 (1)	p =0.99

Table 6.7: Significance of blood rheology and thrombotic mediators on multivariate survival analysis after correcting for the effects of von Willebrand Factor, systolic ankle pressure, sex, and limb sepsis.

The coefficients from this final Cox proportional hazards model were then scaled and rounded to give a risk scoring system as follows:

Score = vWF - (2.5 x ankle pressure) + (115 x sex) + (127 x infection)

(where Sex = 1 if male, 2 if female, and infection = 0 if absent, 1 if present) whereby the higher the risk score, the greater the incidence of death or graft occlusion. Confidence intervals for the relative hazards associated with varying values of the covariates in the final model are shown in Table 6.8 (p.210), and Figure 6.22 (p.211) illustrates Cox model survival curves for differing risk scores.

Graft surveillance: duplex scanning

There were 159 grafts patent at discharge from hospital, however 16 grafts (12 synthetic and 4 vein grafts) occluded prior to the first post-operative duplex scan, 5 patients died during this period, and 2 grafts were lost to follow up. There were therefore 136 grafts (68 synthetic and 68 vein grafts) that entered the program of duplex scanning at 3, 6, and 12 months post-operatively, and a total of 312 duplex examinations have been performed in the course of the study.

Duplex scanning identified 12 vein grafts (18% of those entering surveillance) as being at risk, and in which stenosis (8 grafts) or arteriovenous fistulae (1 graft) were confirmed by angiography. There were 3 grafts that occluded prior to angiography. 2 further vein grafts occluded following a 'normal' duplex scan, while 2 arteriovenous fistulae and a distal in-situ vein graft stenosis were detected in grafts reported as normal on duplex scanning. Overall there were 7 false negative examinations, and 4 false positive examinations in the 68 vein grafts undergoing surveillance (Table 6.9, p.212), giving a sensitivity of 0.63, and a specificity of 0.97 for the use of colour duplex scanning in vein graft surveillance.

In the 68 synthetic grafts undergoing surveillance, there were positive duplex scan findings in 2 of 8 grafts that subsequently occluded, consisting of evidence of popliteal stenosis beyond the graft in one case, and a kink in the graft in the other. In a further graft, thrombus identified within the lumen at the 6 monthly visit (Figure 6.23, p.213), was seen to be resolved at 12 months post-operatively, and there were 3 false positive duplex scans. With a total of 157 scans being performed on synthetic grafts during the study, this gives a sensitivity of 0.33, and a specificity of 0.98 for the use of colour duplex scanning in the post-operative surveillance of synthetic grafts.

VARIABLE	RELATIVE HAZARD FOR	POINT ESTIMATE	95% C.I.
Systolic ankle pressure	L.Q vs U.Q	2.44	1.27-4.66
Sex	Female v male	2.11	1.09-4.10
Infection	yes v no	2.29	1.20-4.37
vWF (iu/dl)	U.Q vs L.Q	1.78	1.34-2.37

Table 6.8: Confidence interval for relative hazards for varying values of covariates in the final model, where L.Q is the lower quartile and U.Q the upper quartile.

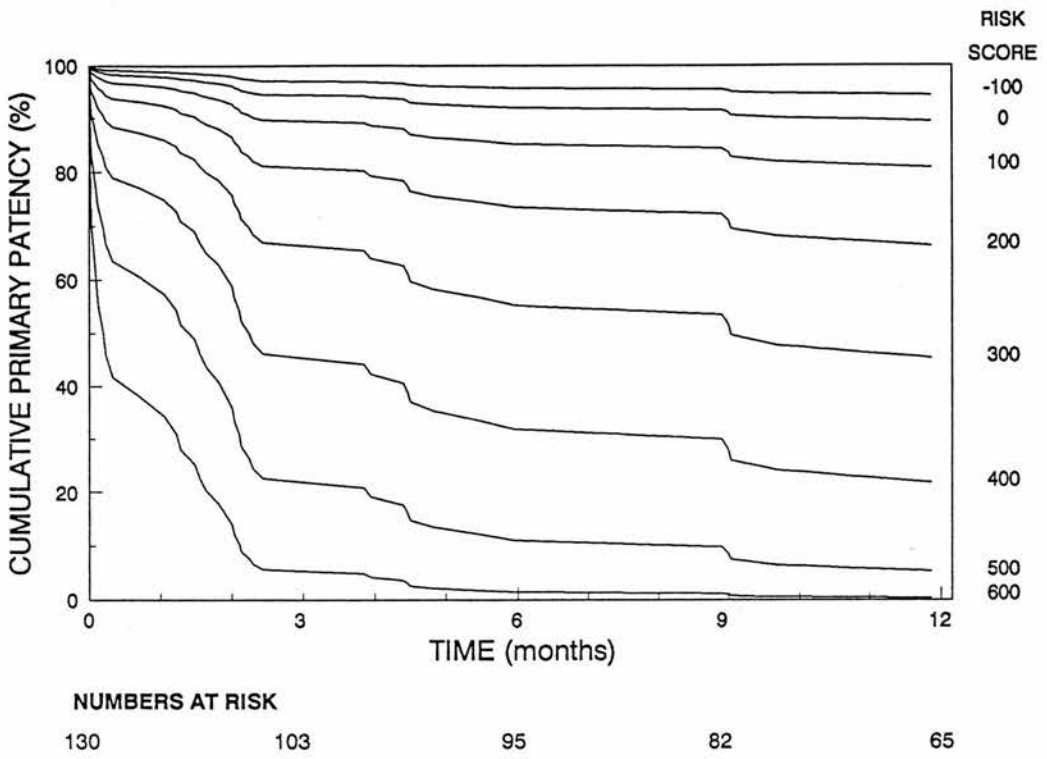


Figure 6.22: Cox model survival curves for infra-inguinal bypass grafts based on the risk assessment score described in the text.

SCAN APPEARANCE	NUMBER OF GRAFTS
Duplex & D.S.A. positives	12
proximal graft stenosis	7
mid graft stenosis	1
arteriovenous fistula	1
mid-graft stenosis	2*
low flow along graft	1*
Duplex positive/D.S.A. negative	4
Duplex negative/D.S.A. positive	7
arteriovenous fistulae	2
run off deterioration	2
distal graft stenosis	1
grafts occluded	2*
Duplex negative/no D.S.A.	132

* indicates grafts in which occlusion occurred prior to angiographic confirmation of non-invasive surveillance results.

Table 6.9: Results of duplex scanning in 68 vein grafts over a 1 year period. All positive scans confirmed by angiography. False negatives identified by angiography performed on grafts identified as "at risk" by other surveillance methods.

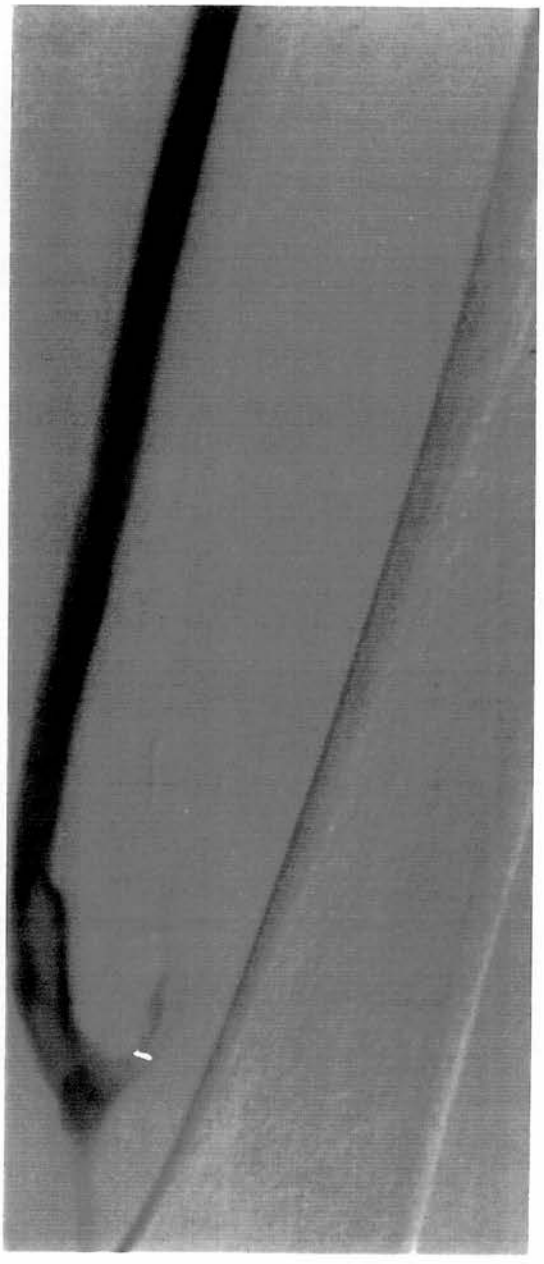


Figure 6.23: Intra-arterial D.S.A. demonstrating thrombus accumulation at the lower end of an above-knee synthetic femoropopliteal bypass graft.

Graft surveillance: Doppler and impedance

There were 49 grafts (28 vein and 21 synthetic) that underwent Doppler insonnation and impedance measurement in addition to colour duplex scanning, and 19 grafts were identified as at risk (Table 6.10, p.215). All grafts confirmed to be "at risk" by angiography had an impedance value above 0.55, with the exception of one graft that occluded prior to angiography (Fig. 6.24, p.216). However this graft had high frequencies on direct insonnation with a 4Khz Doppler probe, and was classed as "at risk" on the basis of this finding.

The sensitivity of impedance measurement in the identification of "at risk" infra-inguinal grafts (vein and synthetic) is therefore 0.95, rising to 1.0 with the addition of direct graft insonnation with a continuous wave Doppler probe, while the specificity of the investigation is 0.91.

Graft material and blood rheology

The median values of rheological parameters in synthetic and vein grafts that remained patent and free from stenosis and run-off deterioration, were plotted against each other to highlight any differences in the haemorrheological response to revascularisation. Haematocrit-corrected blood viscosity (Fig. 6.25, p.217), plasma viscosity (Fig 6.26, p.218), and relative blood viscosity (Fig. 6.27, p.219), followed a similar pattern in both vein and synthetic grafts with median values remaining static, or falling gradually, in the year following surgery, although following insertion of a vein graft plasma viscosity rose transiently before returning to baseline values. Red cell aggregation (Myrenne) fell significantly 3 months following insertion of a synthetic graft (Fig. 6.28, p.220), but thereafter levels were similar in vein and synthetic grafts.

Both white cell count and platelet count were unchanged following revascularisation surgery, with graft material having no effect on the median values over the first post-operative year.

Graft material and thrombotic mediators

Most potential thrombotic mediators were unaffected by the material used as the bypass conduit: Alterations in plasma fibrinogen (Fig 6.29, p.221) following revascularisation were affected by graft type initially, but median values were similar to pre-operative levels by 6 months post-operatively. Levels of von Willebrand Factor (Fig. 6.30, p.222) fell gradually following revascularisation with either synthetic or vein grafts. Plasminogen Activator Inhibitor (Fig. 6.31, p.223), and Factor VII activity (Fig. 6.32, p.224), remained unchanged following

IMPEDANCE FINDINGS	NUMBER OF GRAFTS
Impedance & D.S.A. positive	**18**
run off deterioration	5
grafts occluded	3*
proximal graft stenosis	3
other graft stenosis	3
arteriovenous fistula	3
distal graft stenosis	1
Impedance positive/D.S.A. negative	**12**
Impedance negative/insonnation positive	**1**
graft occluded	1*
Impedance negative/negative or no D.S.A.	**126**

* indicates grafts in which occlusion occurred prior to angiographic confirmation of impedance and graft insonnation results.

Table 6.10: Results of impedance measurement in 49 grafts over a 1 year period. All positive findings based on an impedance value over 0.55 and confirmed by angiography. False negatives identified by angiography performed on grafts identified as "at risk" by other surveillance methods.

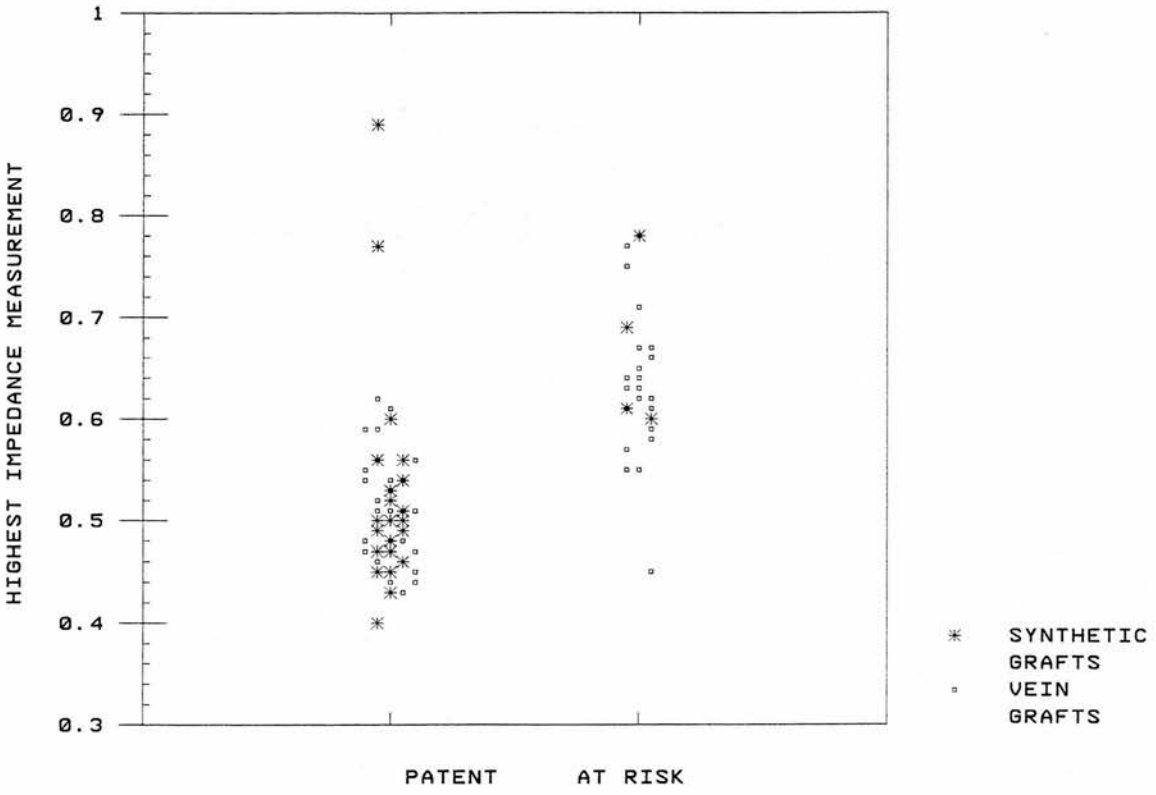


Figure 6.24: Impedance values in grafts remaining patent, and in grafts with angiographically proven stenoses or occlusion.

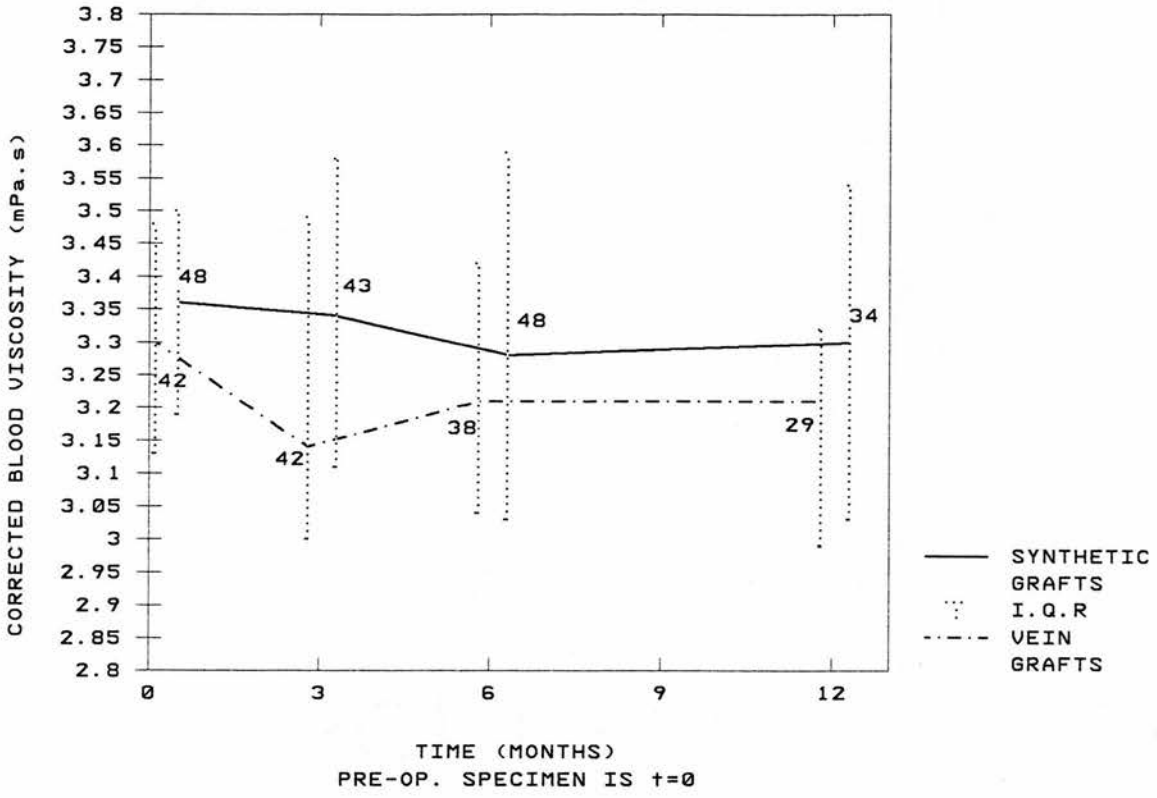


Figure 6.25: Serial changes in median blood viscosity following vein and synthetic infra-inguinal bypass grafting.

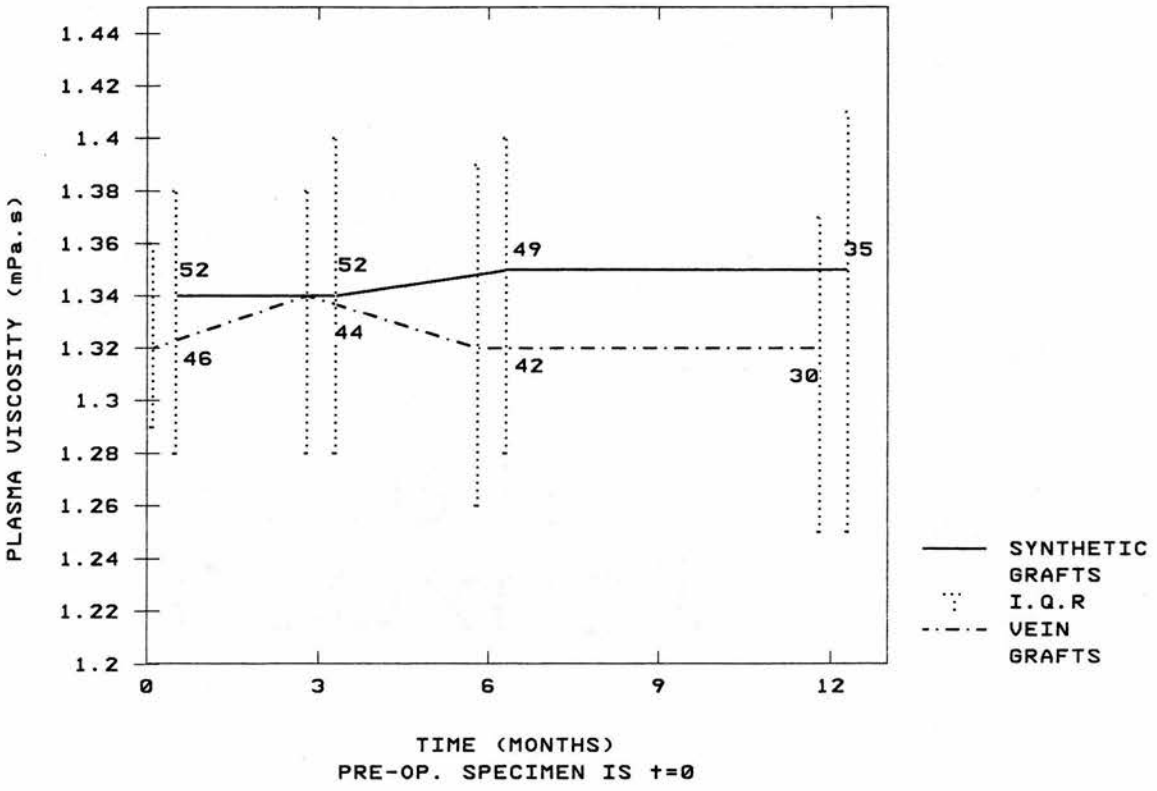


Figure 6.26: Serial changes in median plasma viscosity following vein and synthetic infra-inguinal bypass grafting.

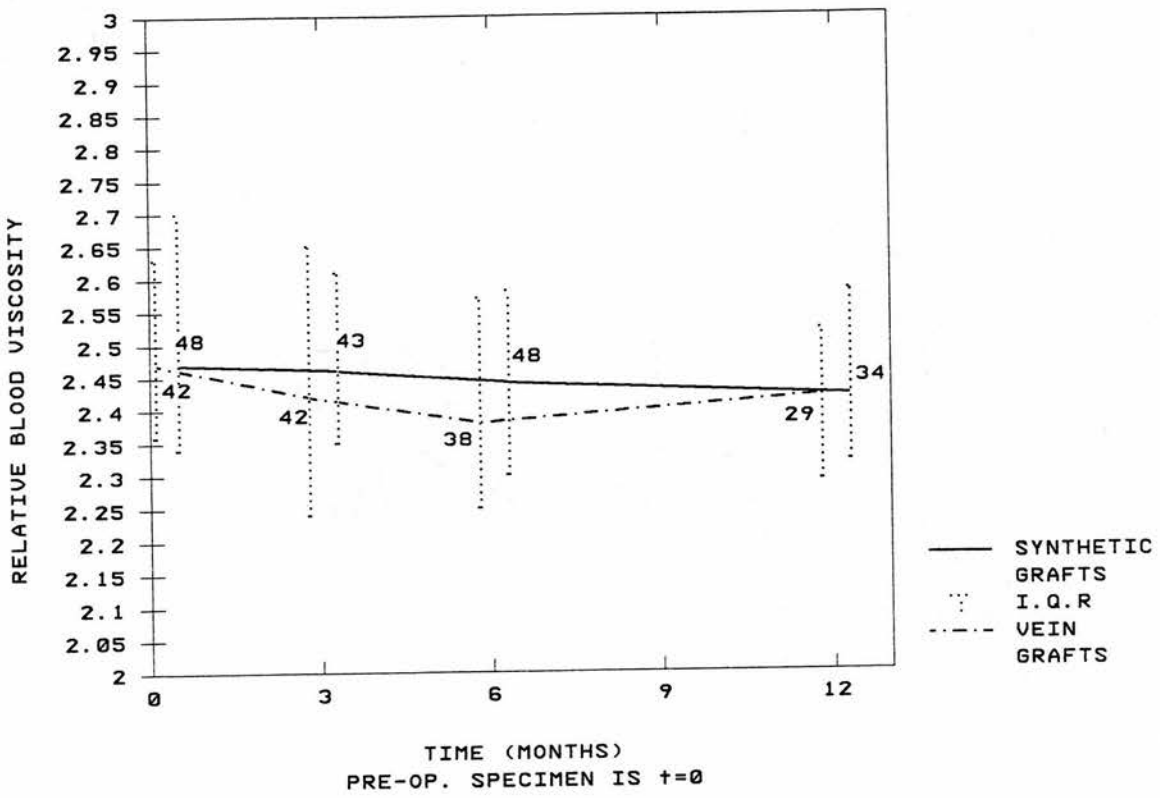


Figure 6.27: Serial changes in median relative blood viscosity following vein and synthetic infra-inguinal bypass grafting.

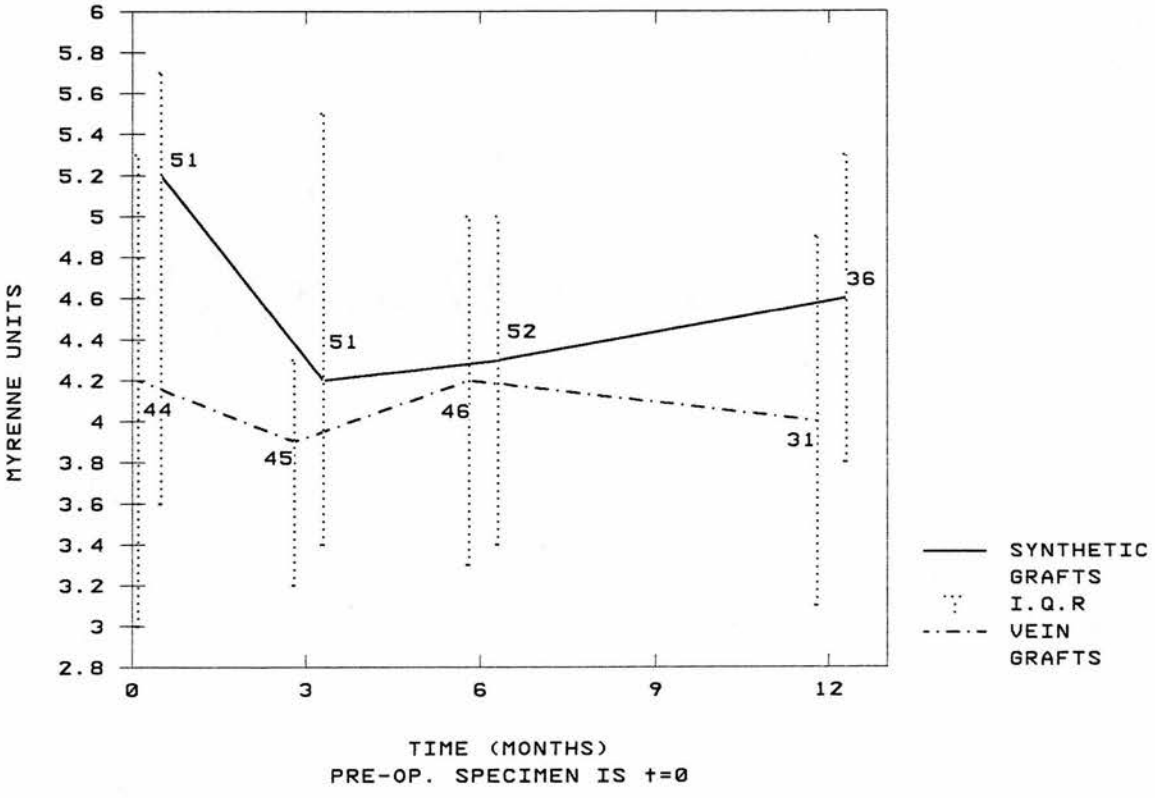


Figure 6.28: Serial changes in median red cell aggregation levels following vein and synthetic infra-inguinal bypass grafting.

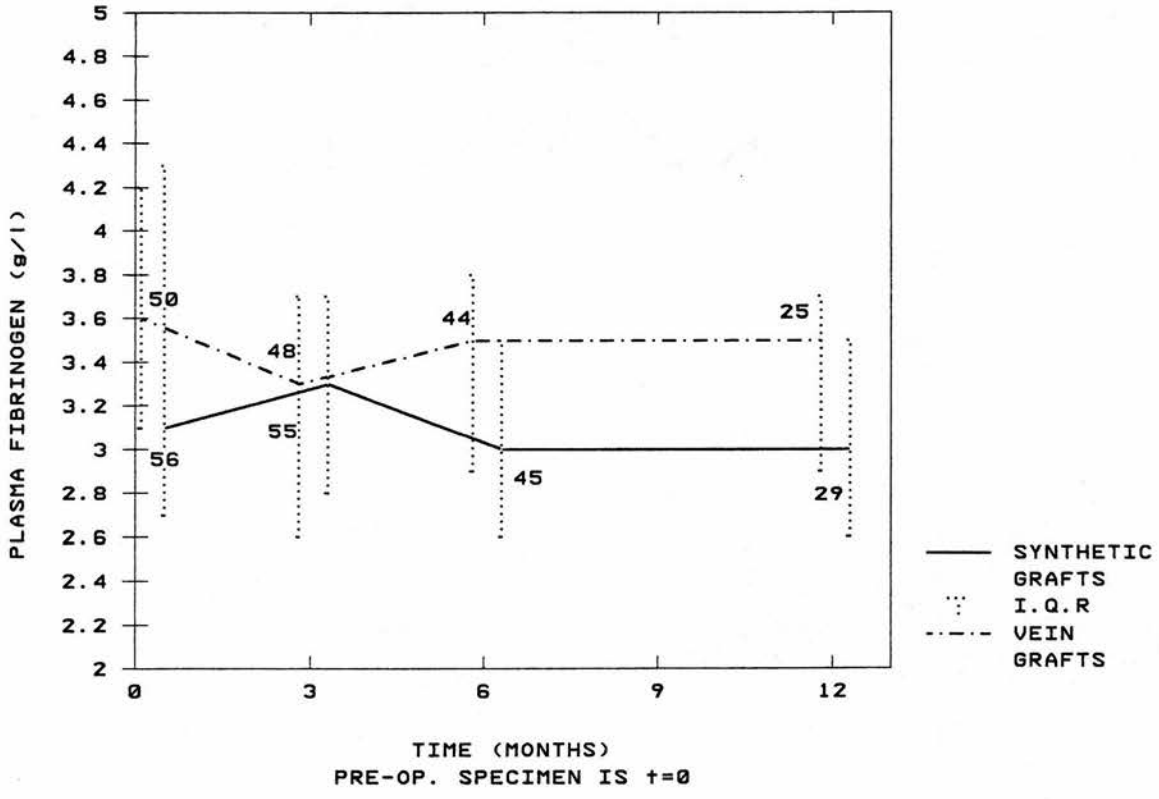


Figure 6.29: Serial changes in median plasma fibrinogen following vein and synthetic infra-inguinal bypass grafting.

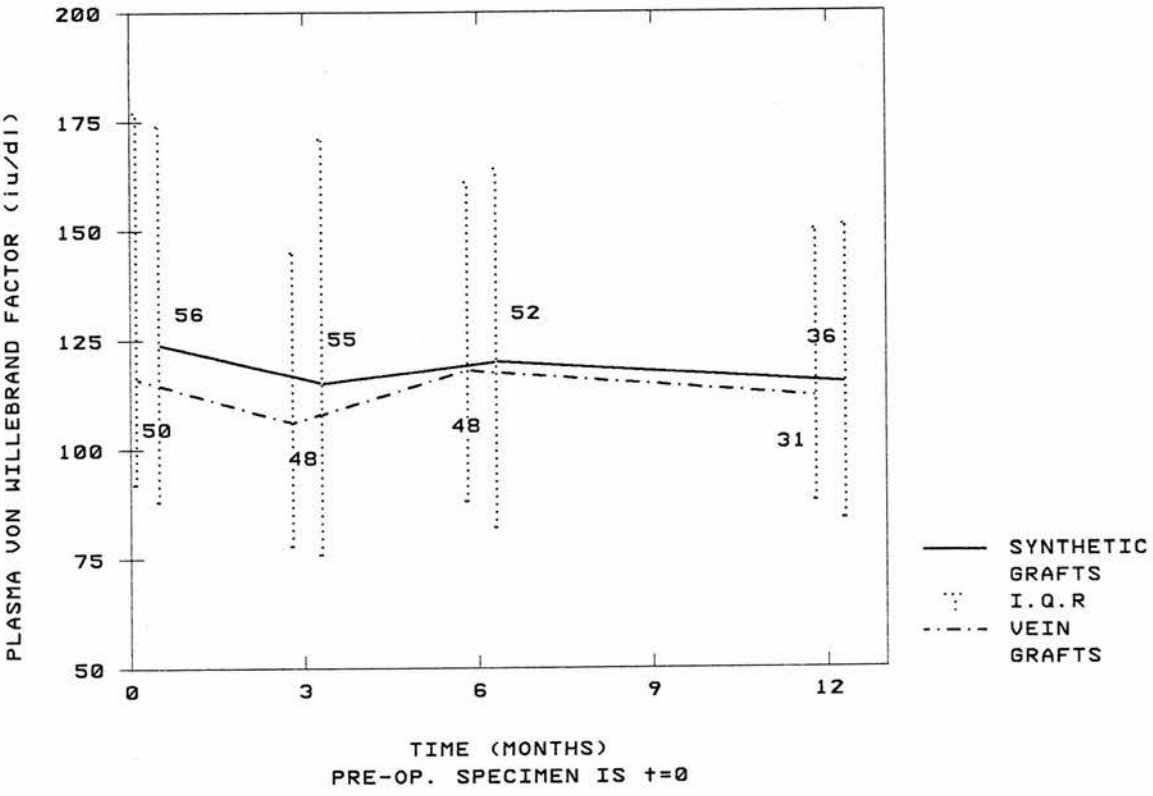


Figure 6.30: Serial changes in median von Willebrand Factor levels following vein and synthetic infra-inguinal bypass grafting.

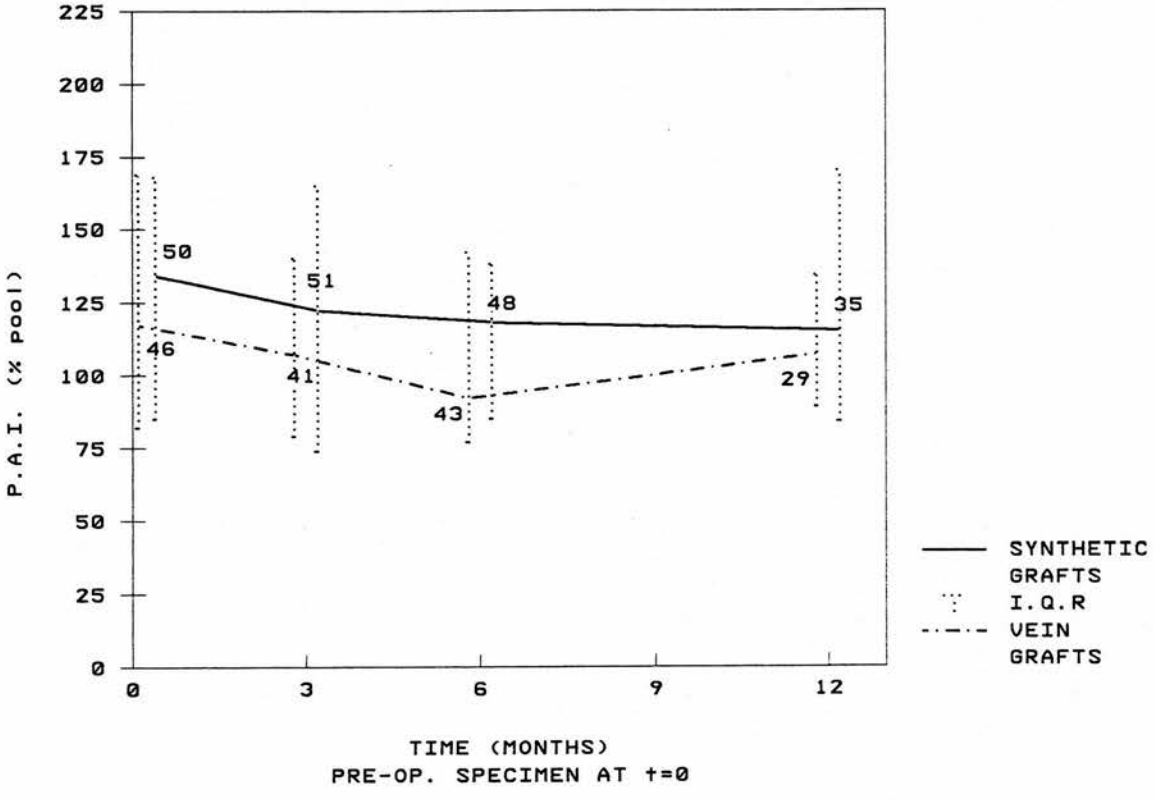


Figure 6.31: Serial changes in median P.A.I. levels following vein and synthetic infra-inguinal bypass grafting.

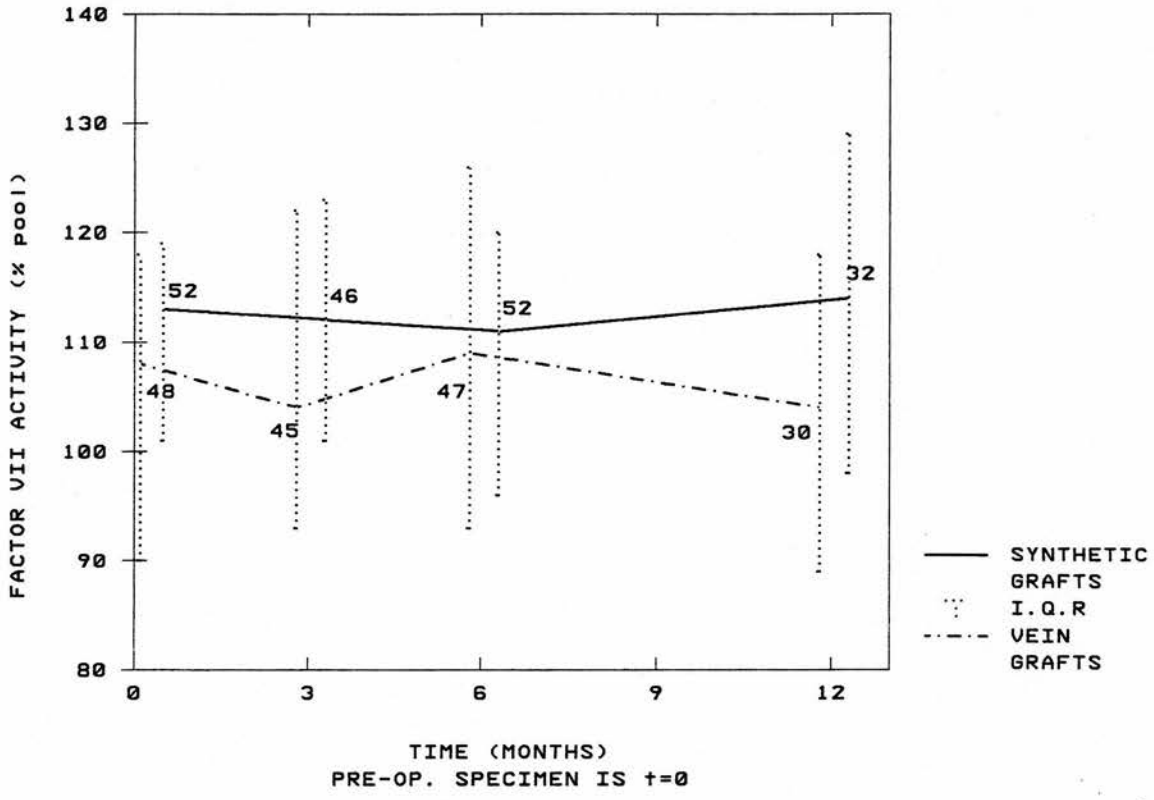


Figure 6.32: Serial changes in median Factor VII levels following vein and synthetic infra-inguinal bypass grafting.

revascularisation, regardless of the graft material used, while tissue Plasminogen Activator (Fig 6.33, p.226), showed a minimal increase following synthetic grafting, remaining unchanged following successful vein grafting.

Post-operative fibrin turnover was greatly affected by the graft material employed in the revascularisation surgery (Fig 6.34, p.227). In the year following insertion of a successful vein graft, the median level of FDP's was slightly reduced over the year, while insertion of a synthetic graft resulted in a marked elevation in FDP's that was apparent 3 months after surgery, and persisted unchanged over the following 9 months.

Post-operative changes and vein graft stenosis

There were 11 vein grafts that were shown to have developed stenoses within the graft in the 6 months following surgery, and in these 11 cases rheological and thrombotic parameters were unchanged from their pre-operative levels at the 3 month assessment (all $p > 0.2$, Wilcoxon matched pairs testing), while in the 46 vein grafts with no evidence of stenosis, run off deterioration, or arteriovenous communications, white cell count ($p < 0.01$), plasma fibrinogen ($p < 0.02$), and von Willebrand Factor ($p < 0.02$) levels, had all fallen significantly 3 months following revascularisation (all Wilcoxon matched pairs testing) (Table 6.11, p.228). However when pre-operative values of these variables are plotted against their values at 3 months post-operatively, there are no significant differences in response between stenosed and normal vein grafts, with the exception of von Willebrand Factor levels (Figure 6.35, p.229). The differences between pre- and post-operative levels of white cell count and fibrinogen in the 2 groups were widely distributed.

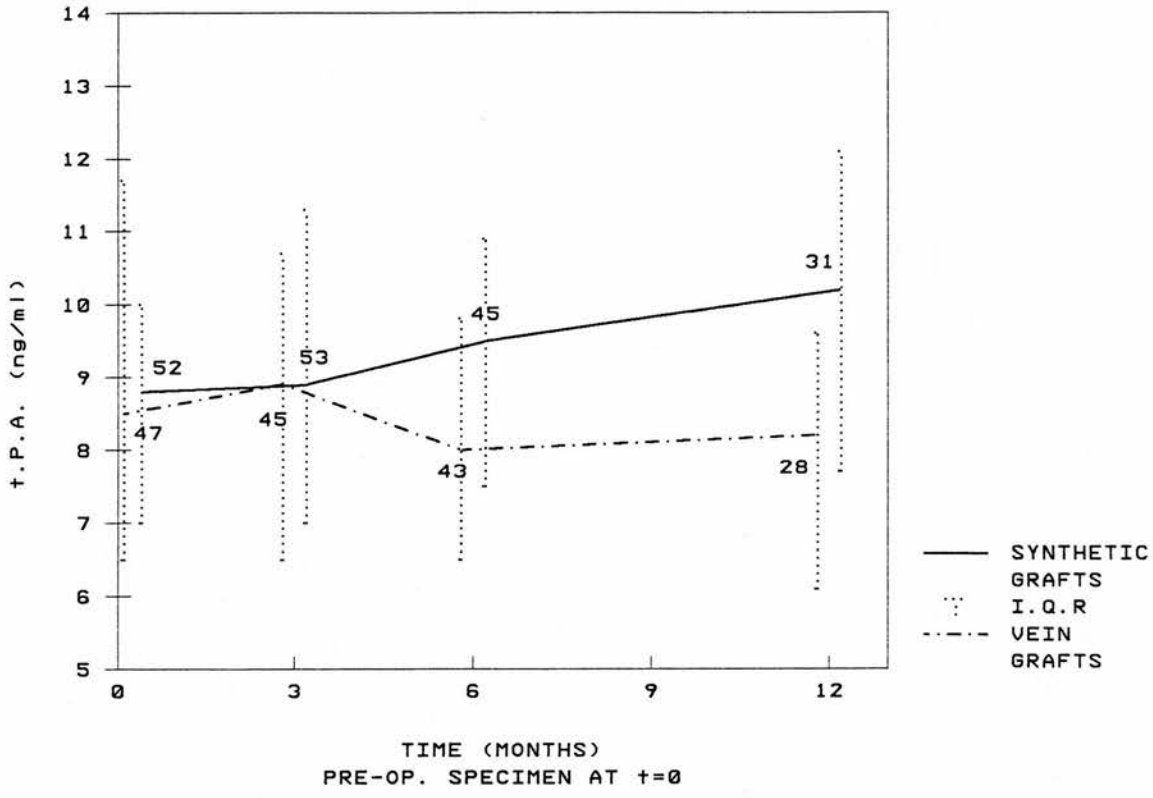


Figure 6.33: Serial changes in median t.P.A. levels following vein and synthetic infra-inguinal bypass grafting.

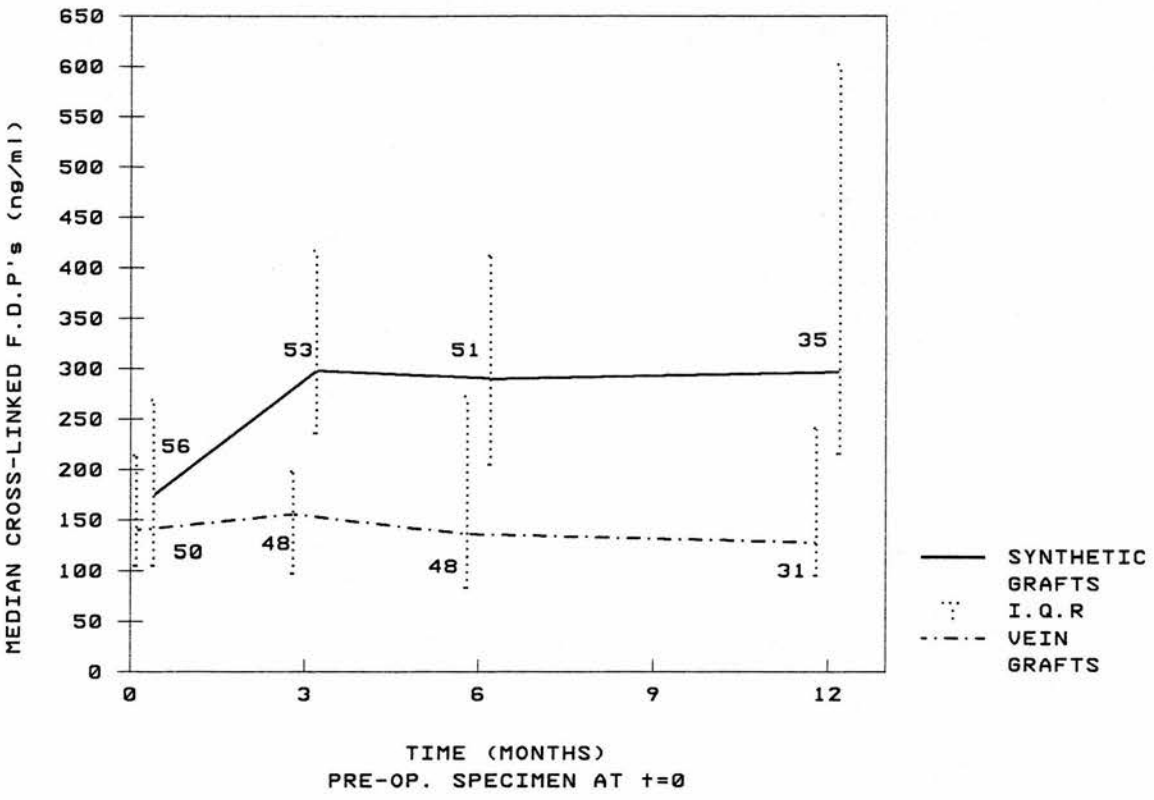


Figure 6.34: Serial changes in median FDP's following vein and synthetic infra-inguinal bypass grafting.

VARIABLE	PRE-OP.	POST-OP.	WILCOXON MATCHED PAIRS
No stenosis			
W.C.C.($x10^9$/l)	8.4 (6.9-9.6)	7.2 (6.5-9.0)	$p < 0.02$
Fibrinogen (g/l)	3.6 (3.0-4.3)	3.3 (2.5-3.7)	$p = 0.02$
vWF (iu/dl)	120 (97-181)	106 (80-151)	$p = 0.02$
Stenosis			
W.C.C.($x10^9$/l)	8.5 (7.2-9.3)	7.2 (6.4-9.0)	$p = 0.29$
Fibrinogen (g/l)	3.6 (2.8-4.7)	3.2 (2.8-4.2)	$p = 0.93$
vWF (iu/dl)	122 (78-180)	148 (125-164)	$p = 0.40$

Table 6.11: Pre- and post-operative white cell count (W.C.C.), plasma fibrinogen, and von Willebrand Factor (vWF) levels in 46 patent and 11 stenosing vein grafts. Figures are medians (interquartile range).

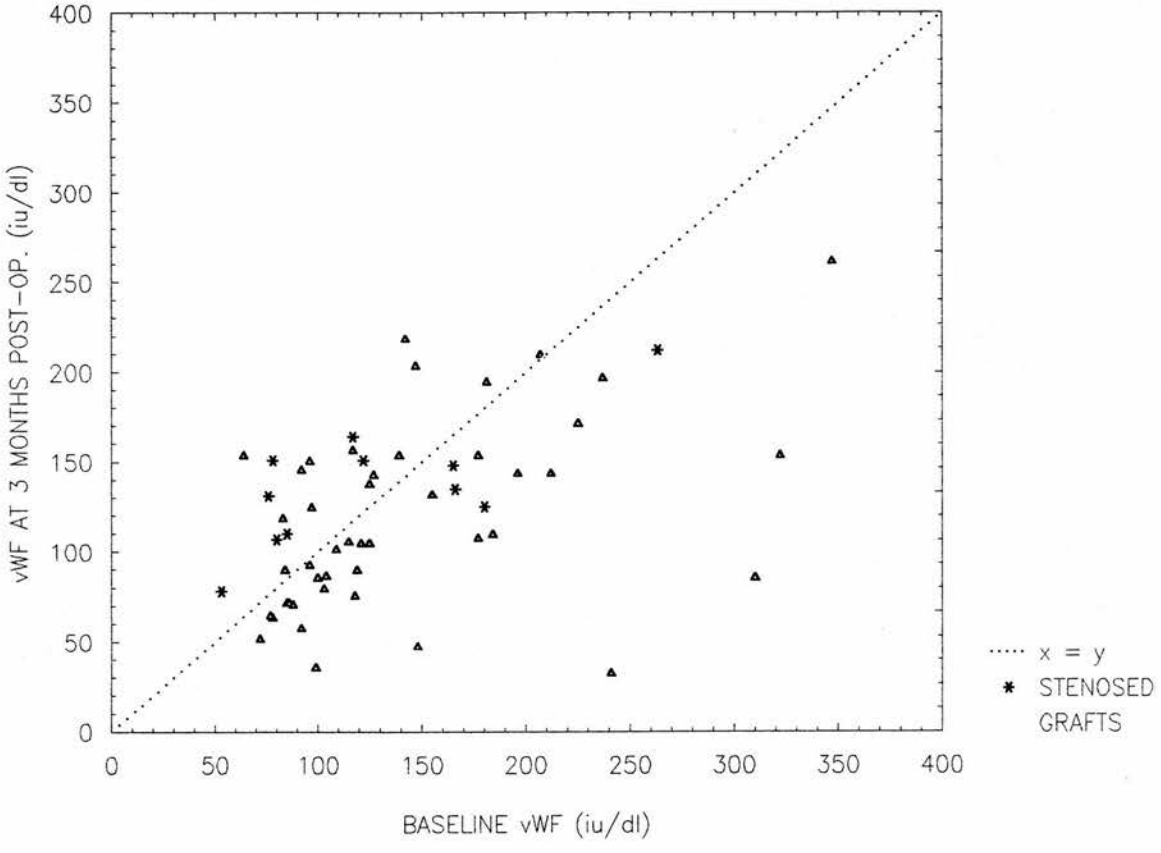


Figure 6.35: Change in von Willebrand Factor levels 3 months after bypass grafting in patent and stenosed vein grafts.

DISCUSSION

Graft and patient survival

The cumulative primary patency rates for this series of consecutive femoropopliteal and femoro-distal grafts, are similar to results reported from other centres (Evans et al, 1981, Hobson et al, 1985, Wiseman et al, 1989, Bergamini et al, 1991, McCollum et al, 1991B), with a patency rate of around 80% for vein grafts, and just below 70% for synthetic grafts. Although some authors claim significantly better results (Leather et al, 1988, Taylor & Porter, 1991, Taylor et al, 1992) these are often based on retrospective studies, and meaningful comparison with results from other centres are hampered by the lack of uniform patient selection and reporting standards (Rutherford & Becker, 1991).

The angiographic disease severity in this study was normally distributed. The results support the data that vein grafts perform better than synthetic grafts, in particular when the anastomosis is to distal popliteal or calf vessels (reviewed by Kempczinski et al, 1990). There were no significant differences in patient characteristics between the 2 graft types in this study to account for the difference in outcome, with an increased number of current smokers in the vein grafts being the solitary significant difference between the 2 groups. Further sub-analyses by type of synthetic material employed are unfortunately rendered of little value by the small numbers in each group.

A 1 year mortality rate of 10% for this group of patients, over one-third of whom were classified as critical limb ischaemia, confirms the high mortality rate consequent on associated cardiac and cerebrovascular disease in patients with PAOD (Norgren, 1990, Dormandy, 1991, Dormandy & Murray, 1991). The 30-day post-operative mortality rate of 6 patients in 186 procedures (3%) is higher than rates quoted by other authors (Harris et al, 1986, Mannick et al, 1991, Cheshire et al, 1992), probably as a consequence of the higher percentage of patients with critical limb ischaemia in this series. Most of the patients dying outwith this 30-day period died within 3 months of successful revascularisation and are therefore unlikely to have obtained significant benefit from the procedure prior to death.

Graft surveillance

Recognition of the need for surveillance of infra-inguinal vein grafts has existed for many years (Breslau & DeWeese, 1965), with up to 30% of such grafts developing asymptomatic fibrous strictures (Szilagyi et al, 1973). In the vein grafts

studied here, 14 (21%) were thought to have developed significant stenoses on non-invasive vascular laboratory tests, 9 of which were confirmed by angiography, and these figures are in keeping with other reported series (reviewed by Varty et al, 1993). Most of these grafts have subsequently undergone successful intervention to maintain graft patency, and this tends to confirm the value of a surveillance program in improving the long-term patency of vein grafts (Harris, 1992). Surveillance of synthetic grafts rarely identified any correctable lesion that led to subsequent graft occlusion, and it seems unlikely that long term patency of infra-inguinal synthetic grafts can be improved by graft surveillance.

The surveillance program confirmed the sensitivity of impedance analysis in the identification of 'at risk' grafts (Wyatt et al, 1991), and the addition of a simple graft insonnation technique improved sensitivity even further. Both these techniques require less capital outlay, and may be easier to learn, than colour duplex scanning, which was again shown to be deficient in the detection of run-off deterioration (Harris, 1992), and was a less sensitive screening technique than has been reported elsewhere (Renton et al, 1991). Both the lack of sensitivity and the surprising inability of colour duplex scanning to identify arteriovenous fistulae in in-situ vein grafts were at least partly due to operator inexperience in the initial stages of the surveillance program, although it would appear that duplex scanning is an unreliable method of assessing run-off vessels in the calf.

The accuracy of the figures for vein graft stenosis derived from these observations is obviously open to question, as only grafts with positive findings on review subsequently underwent direct imaging with angiography or intra-arterial digital subtraction angiography. The incidence of stenosis in grafts with normal findings at surveillance remains unknown in the absence of angiography, and the true false negative rate cannot be calculated. However, grafts have been followed up for up to 1 year post-operatively, and no vein grafts have occluded in the presence of negative surveillance findings. All vein graft occlusions occurred within 6 months of surgery, and it must be assumed that any undetected vein graft stenoses would have declared themselves by graft occlusion within the study period. This assumption rests on the belief that all haemodynamically significant vein graft stenoses ultimately progress to graft occlusion, and this may not be so (DeWeese & Green, 1991). It could be that the incidence of vein graft stenosis in this series is greater than claimed, if a number of "benign stenoses" are escaping detection by non-invasive imaging techniques, although the importance of such strictures is unlikely to be significant in terms of graft outcome.

Two other important conclusions to draw from this surveillance program are the need for graft surveillance to be carried out early in the post-operative course, as a large number of grafts occlude prior to their first surveillance visit at 3 months, and the importance of early angiography following a positive vascular laboratory assessment, as prolonged delay will inevitably lead to occlusion before intervention can be undertaken. Although many of the early graft occlusions occurred in synthetic grafts, there were a number of occlusions in vein grafts that may have been preventable with earlier surveillance and prompt detection of stenoses or flow anomalies.

In summary these studies confirm that post-operative surveillance of infra-inguinal vein grafts leads to increased identification of lesions that may compromise long term patency, thus enabling intervention to prolong patency. This work also suggests that impedance analysis offers a surveillance technique that appears to be more reliable than duplex scanning (Davies et al, 1993), requires less capital outlay, and which, with the addition of a simple Doppler insonnation test, has a sensitivity of 100%. An ideal surveillance program would however include early assessment of grafts prior to hospital discharge, in addition to vascular laboratory assessment at 3, 6, and 12 months post-operatively, with rapid angiography following a positive surveillance visit. These measures should significantly improve the 1-year patency rate for infra-inguinal vein grafts.

Patient characteristics and outcome

A large number of patient and graft characteristics have been shown to affect the outcome of infra-inguinal bypass grafting (Buda et al, 1976, Harris et al, 1987, Rutherford et al, 1988, Tordoir et al, 1993), and this is confirmed on univariate analysis in this study. Many of these variables undoubtedly contribute to graft occlusion for mechanical reasons, with high Bollinger angiogram scores, a low ankle systolic pressure, grafts to below knee vessels, and to single run off vessels, all being measures of the extent of arterial disease, and factors clearly identified as contributing to graft failures (Rutherford et al, 1988, Budd et al, 1990), although not previously associated with post-operative death.

Post-operative mortality was responsible for less than 30% of cases of poor outcome, mortality being attributed to cardiac causes in 50% of cases, a similar rate to that reported elsewhere (Dormandy, 1991), and the association between patient characteristics and mortality is presumably weaker than the association with graft occlusion. There is however evidence that advanced vascular disease is associated with a higher mortality than claudication alone (Norgren, 1990,

Dormandy & Murray, 1991), and other studies have suggested that a low ankle-brachial pressure index is associated with subsequent mortality in patients with peripheral arterial occlusive disease (Dormandy, 1991). The inclusion of post-operative deaths with graft occlusion in the poor outcome category is justified in view of the fact that all but 3 of the deaths occurred within 3 months of surgery, indicating that any benefit from revascularisation was short lived.

Multivariate regression analysis indicated that ankle systolic pressure was the determinant of disease severity with most influence on subsequent outcome, although prior vascular surgery, and emergency revascularisation procedures were also independently associated with poor outcome in an initial model based on patient characteristics alone, their influence on outcome disappearing when thrombotic mediators were added to the outcome model. Thus the assessment of disease severity with the greatest predictive value for the outcome following infra-inguinal revascularisation was the systolic ankle pressure, which is one of the simplest techniques available!

The association between age and poor outcome on univariate analysis could be predicted from the observations in Chapter 3 that disease severity increases with age. Concomitant cardiac disease and post-operative mortality also increase with age (Franco et al, 1990), while the association of female sex with a poor outcome may be a consequence of increased graft occlusion secondary to the smaller vessel diameter in women. Age and female sex remain significant predictors of outcome in multivariate analysis, together with the presence of limb sepsis or tissue necrosis, which in addition to reflecting poor distal circulation, may influence outcome by their tendency to elevate inflammatory reactants and thrombotic mediators such as fibrinogen (Cook & Ubben, 1990, Meade, 1992), therefore increasing the rate of graft occlusion.

Neither smoking habit, nor graft material were significantly related to outcome in this study, in contrast to findings in other series (Wiseman et al, 1989, Budd et al, 1990, Tordoir et al, 1993). The influence of graft material however appeared to be confined to grafts to infragenicular vessels (Rutherford et al, 1988), which accounted for a minority of cases in this study, and although no significant difference was observed in terms of outcome, graft patency was greater for vein grafts at all levels of anastomosis. The lack of any smoking-related effect on outcome may be consequent upon small numbers of non-smokers, although other studies have also failed to find a significant relationship between smoking habit and graft patency (Budd et al, 1990).

In summary, although several previously identified risk factors for graft occlusion were found to be significantly associated with a poor outcome following infra-inguinal bypass grafting on univariate analysis in this study, the only factors that remained significantly associated with poor outcome following multivariate analysis were female sex, the presence of limb sepsis, advanced age, and a low ankle systolic pressure in the affected limb. Some of these factors may be detrimental to the outcome of infra-inguinal revascularisation surgery by virtue of their tendency to increase levels of potentially harmful thrombotic mediators such as fibrinogen, and further studies investigating the effects of reducing plasma fibrinogen levels in these patients are required.

Blood rheology and outcome

There did not appear to be any relationship between blood rheology and the outcome of revascularisation surgery in this study, despite the presence of various mechanisms by which abnormal blood rheology could contribute to graft occlusion (Lowe, 1986, Lowe, 1992, Koenig & Ernst, 1992). Reduced haematocrit and low haemoglobin were related to a poor outcome on univariate analysis, but not when other variables were taken into consideration, while an elevated platelet count appeared to be independently associated with a poor outcome until potential thrombotic mediators were also considered in the predictive model.

The link between elevated platelet count and a poor outcome was reinforced by the observation that post-operative antiplatelet therapy (aspirin) is associated with a good outcome. This observation failed to reach statistical significance, but is similar to the findings of other studies that have examined the effects of antiplatelet therapy on infra-inguinal graft patency (McCollum et al, 1991A). Although these studies have failed to prove conclusively that post-operative aspirin therapy improves graft patency there is however evidence that antiplatelet therapy reduces vascular mortality in patients with a history of cardiac and cerebrovascular disease (Antiplatelet trialists collaboration, 1988), as well as in peripheral vascular disease (McCollum et al, 1991A).

The univariate associations of low haematocrit and haemoglobin, and elevated white cell count, with a poor post-operative outcome may reflect the effect of limb sepsis and tissue necrosis on these variables: Infection and tissue necrosis promote a leucocytosis (Walter & Israel, 1987D), while the low haemoglobin and haematocrit reflect the haematological stress response (Reizenstein, 1979) to chronic sepsis. The value of an elevated pre-operative white cell count as a predictor of femoropopliteal graft failure has recently been confirmed (Thomas et

al, 1993). If these increases in white cell count are associated with increased levels of white cell activation products, which have been shown to be both toxic to vascular endothelium (Sacks et al, 1978, Smedly et al, 1986) and to have a vasoconstrictive action on atherosclerotic vessels (Lopez et al, 1989, Mügge et al, 1991), then this could be the means by which limb sepsis and tissue necrosis promote a poor outcome following revascularisation surgery, and this merits further investigation.

Thrombotic mediators and outcome

The observation that levels of potential thrombotic mediators are related to poor outcome following revascularisation surgery has not been reported previously, with the exception of the association between elevated fibrinogen and subsequent outcome following revascularisation (Hamer et al, 1973, Harris et al, 1988, Wiseman et al, 1989). The association of elevated fibrinogen with graft occlusion may reflect a mechanism by which other risk factors, such as smoking, act to bring about graft occlusion (Wiseman et al, 1989), a belief supported by the observation that elevated fibrinogen is not independently associated with poor outcome in this study.

The elevation in fibrinogen in poor outcome cases in this study may partly reflect its correlation with disease extent, and is partly a consequence of infection, which is known to increase fibrinogen levels (Cook & Ubben, 1990), probably via the production of interleukin-6 by monocytes (Ritchie et al, 1982, Reid, 1991). The highly significant independent association of pre-operative limb sepsis with a poor post-operative outcome that was observed in these studies, may be a result of the associated elevation in plasma fibrinogen which may predispose to thrombosis by increasing atheroma (Smith et al, 1990, 1992), increasing fibrin formation (Meade, 1992), and increasing platelet aggregation (Meade, 1992). All of these mechanisms could ultimately lead to either graft occlusion, or cardiac events secondary to coronary artery occlusion, and current evidence indicates that plasma fibrinogen may play a significant role in both these events (Wiseman et al, 1989, Meade, 1992).

The association of elevated FDP's with a poor outcome on univariate analysis is partly a measure of the relationship between disease severity and outcome following revascularisation surgery. It has previously been demonstrated that FDP levels are independently predictive of the angiographic severity of arterial disease (Chapter 3), and it may be that the association between elevated FDP levels and poor outcome observed in this study is a consequence of the associations

between markers of increased disease severity and a poor outcome. In this respect FDP's may be merely another indicator of the extent of arterial disease, although their predictive capacity would appear to be of little significance alongside systolic ankle pressure in the final model.

Low factor VII levels were also associated with a poor outcome on univariate analysis, a finding previously observed in a multicentre study which showed associations between low factor VII levels, and both graft occlusion and myocardial infarction on univariate analysis (Cortellaro et al, 1992). In this study the associations also disappeared on multivariate analysis, and it is likely that any association between factor VII levels and graft outcome may be a consequence of infection reducing factor VII levels (Meade, 1991).

von Willebrand Factor is a plasma glycoprotein that has been shown to be an essential requirement for the development of occlusive thrombi at sites of arterial injury (Brinkhous et al, 1991), and an association between elevated vWF levels and poor outcome following infra-inguinal grafting may indeed be causal: vWF is important in platelet adhesion to subendothelium at sites of vessel injury, and the mechanism of platelet adhesion and activation is dependent on the presence of vWF, such that if vWF is absent, mural thrombi will not form at sites of injury (reviewed by Badimon & Fuster, 1992).

Elevated levels of vWF may therefore lead to increased platelet adhesion and thrombus formation within diseased native vessels, perhaps promoting graft occlusion by compromising inflow and outflow vessels. Anastomotic sites may also be prone to increased thrombus formation, as these are areas where endothelial injury occurs at the time of surgery, and also sites of increased shear rates due to the anatomical arrangement of the end-to-side anastomosis. Increased shear rates at sites of arterial injury are thought to contribute to increased platelet deposition (Badimon & Fuster, 1992), and this may be more marked in the presence of increased plasma vWF.

Elevated pre-operative vWF levels may therefore theoretically lead to an increased rate of occlusive intravascular thrombus, with subsequent graft occlusion or cardiac mortality, and the findings in this study of an independent association between elevated vWF levels and poor post-operative outcome (both death and graft occlusion) would support this belief.

This association between elevated vWF levels and poor outcome following infra-inguinal revascularisation may be amenable to pharmacological manipulation to maintain graft patency: Although post-operative antiplatelet agents may be of some benefit in peripheral arterial disease (Antiplatelet Trialists Collaboration,

1988, McCollum et al, 1991A), presumably as a consequence of the role of platelets in thrombus formation (reviewed by Smith, 1991), it may be that in the presence of abnormally high vWF levels, platelet adherence and aggregation (and subsequent thrombus formation) occur without the requirement for Thromboxane A2 release. This would account for the limited effects of antiplatelet therapy on post-operative graft patency, as aspirin therapy has no effect on vWF levels (Reid, 1991). An alternative approach, aimed at reducing vWF levels, may therefore be required to improve graft patency. Such an approach could involve the use of monoclonal antibodies to vWF, currently used in experimental models to investigate the role of vWF in thrombogenesis (Badimon & Fuster, 1992), and further studies are required to assess the value of this form of therapeutic intervention.

In the absence of any currently available pharmacological means of improving the outcome of infra-inguinal bypass grafting apart from antiplatelet therapy, an alternative is to improve the results of such surgery by improving pre-operative patient selection. The use of a risk scoring system that considers thrombotic mediators in addition to patient characteristics (Budd et al, 1990), may offer a more accurate method of assessing the suitability of an individual for infra-inguinal revascularisation procedures, although the scoring system developed from these studies requires further prospective validation before it can be used to assist in patient selection.

In summary, a number of potential thrombotic mediators were elevated pre-operatively in cases of infra-inguinal bypass grafting with a poor outcome, and these elevations may promote increased thrombus formation. Only elevated von Willebrand Factor levels were independently related to poor outcome, and this may be a consequence of its role in platelet aggregation and thrombus formation. Increased levels of fibrinogen and fibrin turnover may be the means by which other independently predictive risk factors, such as limb sepsis and disease severity, contribute to graft occlusion and death.

These findings offer the potential for future pharmacological therapy, but at present the use of a pre-operative risk scoring system incorporating vWF offers the best means of improving the results of infra-inguinal revascularisation surgery by identifying patients unlikely to benefit from attempts at reconstructive surgery.

Graft material & blood rheology

There appear to be few major changes in rheological variables in the first year following successful infra-inguinal revascularisation surgery. This suggests that the alterations in blood rheology that are observed in patients with PAOD, is not a consequence of tissue ischaemia alone, as successful revascularisation fails to reverse the changes in blood rheology.

The graft material employed does not appear to influence blood rheology over the first post-operative year, with the pre-operative differences in the 2 graft material groups being maintained at subsequent sampling, and median values remaining almost constant over the period of the study.

Graft material and thrombotic mediators

Most potential thrombotic mediators are unaffected by the graft material employed, and show little change from their pre-operative levels in the year following revascularisation, despite transient alterations in the early post-operative period that are probably related to the resolution of limb sepsis, and the acute phase response that accompanies surgery.

This relative stability in levels of thrombotic mediators following surgery indicates that successful infra-inguinal revascularisation does little to reverse the potentially thrombogenic abnormalities observed in patients with PAOD. This may be a consequence of post-operative improvements in thrombotic parameters being offset by progression of arterial disease following revascularisation, or a reflection of a genetic influence on the levels of some variables such as fibrinogen (Humphries et al, 1987, Fowkes et al, 1992), which maintains potential thrombotic mediators at an abnormally high level.

Synthetic infra-inguinal grafting appears to result in a prolonged increase in fibrin turnover, and elevated FDP's are found for at least 12 months, indicating that the fibrin layer found within synthetic grafts (DeBakey et al, 1964) is a dynamic layer that is constantly being remodelled and altered. This is further supported by the observation that t.P.A. levels rise, and P.A.I. levels fall, in the period following synthetic grafting. Overall this indicates a shift in favour of fibrinolysis, and this is probably required to lyse the fibrin that is continuously deposited within the graft. Subsequent lysis of this fibrin will prevent occlusion and further studies will be required to determine whether or not the level of fibrin turnover in synthetic grafts is related to long-term patency.

It is notable that analysis of the small number of vein grafts in whom stenoses were detected by the surveillance program, indicates that plasma

fibrinogen and von Willebrand Factor levels remained elevated following revascularisation, in contrast to cases where the vein graft remained patent and free from stenoses in the initial post-operative year. As already discussed, vWF is required for platelet adhesion and thrombus formation (Smith, 1991, Badimon & Fuster, 1992), while fibrinogen has been identified in atherosclerotic plaques (Bini et al, 1989), and although the pathogenesis of vein graft stenosis is unclear (Varty et al, 1993), the observations made in these studies may be of relevance to the aetiology of vein graft stenosis. In view of the small numbers of cases studied, and the limitations of the graft surveillance program, more detailed investigation is however required to determine the significance of these observations.

Summary

Elevated levels of von Willebrand Factor, a glycoprotein with a role in platelet-vessel wall interaction, have been shown to be independently associated with a poor outcome following infra-inguinal revascularisation, and a risk scoring system which takes this and other pre-operative patient characteristics into consideration has been developed to assist in pre-operative patient selection.

Elevation in vWF levels may play a role in the pathogenesis of infra-inguinal graft occlusion through its effects on platelet aggregation, while the effects of other patient characteristics that are independently associated with outcome (ankle pressure, sex, infection) may be mediated in part by elevated levels of other potential thrombotic mediators such as fibrinogen.

Blood rheology and thrombotic mediators are not significantly altered following successful revascularisation surgery, although there is some evidence from these studies that the response to surgery may be influenced by the graft material employed, and by the development of post-operative vein graft stenoses. Further studies are required to clarify whether or not these observations are causal in nature.

The use of a non-invasive graft surveillance program has been confirmed to be of value in the detection of vein graft stenosis, and should be commenced early in the post-operative course. The use of computer-assisted impedance measurement offers a reliable and inexpensive alternative to colour duplex scanning in vein graft surveillance, and this finding has not previously been reported in a prospective study. These studies however, failed to identify any technique that was of value in the identification of 'at risk' synthetic grafts.

CHAPTER 7

Discussion

Blood Rheology and disease severity

The absence of any significant change in plasma viscosity and red cell aggregation in cases with peripheral arterial occlusive disease, reported in this thesis, together with the significant reduction in blood viscosity and relative blood viscosity, contradict previously reported findings in patients with symptomatic peripheral arterial occlusive disease (Dormandy et al, 1973A, Ernst & Matrai, 1987, Reid, 1991). This may reflect the nature of the control population selected for comparison, which was a random population sample, rather than an age-matched group of disease-free patients, but is more likely to be related to the severity of the PAOD in the patient group studied.

Previous studies of blood rheology in arterial disease have concentrated on patients with mild to moderate claudication (Dormandy et al, 1973A), implying a skewed distribution of disease severity in favour of mild disease. This contrasts with the Gaussian distribution of angiographically assessed disease severity in the patient population studied in this thesis, all of whom had disease severe enough to merit consideration for reconstructive surgery, that is, moderate to severe claudication, or rest pain and critical limb ischaemia. The studies in patients with critical limb ischaemia (Chapter 4) indicate that severe arterial disease is associated with a reduction in plasma proteins, and while it is possible that plasma viscosity is increased in patients with mild arterial disease, the effects of advanced PAOD appear to favour a reduction in plasma proteins, the net effect being that there is no significant difference between the median plasma viscosity in the patients studied, and that in age-matched population controls.

The findings of a reduced blood viscosity in addition to other features of a haematological stress response (Reizenstein, 1979) to chronic peripheral arterial occlusive disease suggest that the rheological changes observed in PAOD are a consequence of the biochemical and haematological responses to the disease state, while the observations made in previous studies reflect the effects of an increase in fibrinogen on blood and plasma viscosity (Reid, 1991). In more advanced PAOD the effects of fibrinogen on viscosity are offset by the changes in plasma proteins that accompanies the haematological stress response.

The belief that the rheological changes found in association with PAOD are a response to the disease process is further supported by the lack of any independent associations between rheological parameters and angiographic severity of disease in the patients studied, and by the observation that revascularisation of a critically ischaemic limb results in a normalisation of rheological parameters (Chapter 4).

There is now an increasing body of evidence that reversible tissue ischaemia is associated with a number of biochemical events leading to cellular damage and an inflammatory response (Shearman et al, 1988, Hickey et al, 1990, Adiseshiah et al, 1992, Hickey et al, 1993) and these may precipitate alterations in blood rheology as a consequence of the release of inflammatory mediators. Many of these events are thought to be mediated by neutrophil products (Romson et al, 1983, Neumann et al, 1990, Welbourn et al, 1991), and levels of the monocyte product IL-6 are elevated in cases of peripheral arterial disease (Reid, 1991). It is therefore important to investigate white cell activation in future clinical studies in peripheral arterial disease by measuring markers of neutrophil activation (e.g. elastase) and monocyte activation (e.g. interleukin-6), and determining the relationship between these products, blood rheology, and arterial disease.

Thrombotic mediators and disease severity

This thesis has demonstrated that levels of cross-linked fibrin degradation products (FDP's) are independently associated with the angiographic severity of occlusive arterial disease, and that plasma fibrinogen and von Willebrand Factor (vWF) show a trend towards significant independent association on multivariate analysis. These associations between potential thrombotic mediators, and a direct assessment of disease severity, have not been reported previously.

The confirmation that levels of potential thrombotic mediators are elevated in comparison with age-matched population controls, provides further evidence that the biochemical environment in patients with occlusive arterial disease is capable of promoting increased thrombus formation. The presence of increased fibrin turnover reflects increased thrombus formation that may result in an increase in atherosclerosis (Thompson et al, 1990, Lorenzet et al, 1992), while the accompanying elevations in plasma fibrinogen and vWF will further increase thrombus formation (Brinkhous et al, 1991), with the elevation in P.A.I. levels resulting in inhibition of fibrinolysis. Despite failing to show independent correlation with disease severity, elevations in fibrinogen and vWF may be one means by which age and limb sepsis influence the severity of arterial disease, with advancing age increasing fibrinogen levels (Meade, 1992), while vWF has some of the characteristics of an acute-phase reactant (Smith, 1991).

The existence of a prothrombotic environment in patients with occlusive arterial disease is maintained after resolution of critical limb ischaemia (Chapter 4), indicating that associations between potential thrombotic mediators and disease severity are not a solely a consequence of tissue ischaemia. The post-

revascularisation fall in plasma fibrinogen, vWF, and t.P.A. levels, results from the resolution of limb sepsis, as these mediators are increased in inflammatory conditions (Smith, 1991, Meade, 1992), but their failure to return to normal population levels implies that additional factors influence plasma levels of these potential thrombotic mediators. These are probably related to cigarette smoking (Wiseman et al, 1989, Reid, 1991, Blann, 1991, 1992) and in the case of fibrinogen, an additional genetic effect (Thomas et al, 1991, Fowkes et al, 1992), although further studies into fibrinogen genotype are required. The persisting prothrombotic state following revascularisation surgery has not previously been reported and has important implications for the longer-term results of surgery. Studies into the use of pharmacological therapies aimed at lowering levels of potential thrombotic mediators (Meade, 1992) in patients with PAOD, are therefore required.

Percutaneous angioplasty

This procedure has become the mainstay of treatment in claudication, and has also been recommended in critical limb ischaemia (Lu et al, 1982). The studies undertaken for this thesis confirm that in appropriate cases the short-term success rate of angioplasty is excellent. The comparison of venous and peri-lesional arterial samples refutes the suggestion (Schwartz et al, 1989) that local alterations in haemorrheological parameters are responsible for the development of atherosclerotic lesions at specific sites, and this has not previously been reported.

In view of the tendency for patients undergoing angioplasty to have less severe arterial disease than patients undergoing reconstructive surgery, it is not surprising that angioplasty produces few changes in rheological parameters, which remain similar to those encountered in age-matched controls both prior to and following angioplasty. This suggests that the biochemical changes associated with a chronic disease state are not found in the presence of the less severe arterial occlusive lesions typically selected for angioplasty, and is further evidence that rheological alterations in claudicants are secondary to biochemical changes that accompany advanced PAOD.

The immediate effects of angioplasty on intra-arterial thrombotic mediators mirror the pathophysiological changes that have been demonstrated to result from balloon angioplasty (Block et al, 1980, 1981, Castaneda-Zuniga et al, 1980, Brady & Warren, 1991), with changes in thrombotic mediators that reflect endothelial damage and fibrin deposition at the site of angioplasty. These changes are however

transient, and successful angioplasty produces no long term alterations in levels of thrombotic mediators, with the sole exception of cross-linked FDP's.

The persisting elevation in fibrin turnover (FDP's) that accompanies successful angioplasty may be required to prevent thrombus formation at the site of angioplasty leading to restenosis. This is supported by the observation that fibrin turnover remains unchanged in cases with evidence of restenosis following angioplasty, although only a small number of patients fell into this category. Alternatively, in view of the independent association of FDP's with the angiographic severity of disease, the increase in FDP levels following angioplasty may indicate progression of arterial disease (Fowkes et al, 1993) as a consequence of successful angioplasty. There is experimental evidence that subcritical stenoses enhance distal arterial disease (Bomberger et al, 1981), and the endothelial injury and fibrin formation occurring at sites of angioplasty, may result in such a stenosis, which could promote distal disease progression. A more likely explanation of this finding however, is that successful angioplasty restores normal flow to the arterial tree distal to a stenosis. This will increase contact between atherosclerotic distal vessels and blood, with a subsequent increase in fibrin turnover as fibrin is deposited in the vicinity of atheromatous plaques.

Recent evidence suggests that elevations in FDP levels are also predictive of subsequent coronary events (Fowkes et al, 1993), although this is not proven to be a causal association. It remains to be seen whether or not the increase in FDP levels following angioplasty increases the coronary risk in patients with PAOD.

These potentially detrimental effects of angioplasty, and the possible role of fibrin turnover in restenosis following angioplasty, require to be studied in more detail in a much larger patient population, with a longer period of follow-up, ideally with angiographic studies to determine outcome more accurately.

Infra-inguinal bypass grafting

These studies have confirmed that the results of femoropopliteal and femoro-distal grafting are poorer than for more proximal procedures, and that up to one-quarter of vein grafts will develop stenoses within 6 months of surgery. These findings confirm the value of a graft surveillance program (Harris, 1992). Analysis of the results of duplex scanning and impedance measurement indicate that both techniques will adequately detect graft stenoses, with impedance measurement having the added advantage of assessing distal run-off in addition to the graft itself. The adoption of a graft surveillance program based on either of these techniques, with angiography reserved for grafts identified as "at risk" on initial screening, will

enable pre-emptive treatment of graft stenoses, thus improving the long-term graft patency without the risks and expense of routine angiographic imaging in all infra-inguinal grafts.

Having demonstrated a strong association between potential thrombotic mediators and disease severity, the results in this thesis have also indicated that many thrombotic mediators have a role in graft occlusion and post-operative death. Of particular relevance is the strong independent association of elevated vWF with graft occlusion and death. The influence of other patient characteristics such as age, and limb sepsis on poor outcome are possibly a consequence of the increased levels of fibrinogen and other potential thrombotic mediators which accompany these conditions, and which were elevated in cases with a poor outcome.

The link between vWF and graft occlusion may be causal, with evidence from animal studies that vWF is an essential requirement for the development of occlusive thrombi, having a major role in platelet-endothelial adhesion at high shear rates (Badimon & Fuster, 1992). High shear rates are typically encountered at vascular anastomoses. It is therefore possible that graft failure is a consequence of increased vWF levels which are present prior to surgery, and which may increase further in response to surgery (Smith, 1991). The effects of increased vWF levels on intravascular thrombus formation have not been directly studied, although circumstantial evidence of an increased thrombotic tendency in association with elevated vWF does exist (Zwaginga et al, 1990). The clinical effects of reducing vWF levels on graft patency would provide more substantial proof of the role of vWF in graft occlusion, and such studies should be performed.

Before these studies can be performed however, a suitable method of reducing plasma vWF must be found. The effects of currently available antithrombotic agents on vWF levels have not been studied, with the exception of low dose warfarin (Reid, 1991), although post-operative anticoagulation appears to improve post-operative survival following femoropopliteal bypass grafting (Kretschmer et al, 1988), and merits further investigation in this situation, as does the use of low-molecular weight heparins. An alternative approach would be to use monoclonal antibodies against vWF, which are effective in reducing vWF levels in experimental animal models (reviewed by Badimon & Fuster, 1992), and studies aimed at assessing the effects of these and other antithrombotic agents on both thrombotic mediators and graft survival, are required. Such studies of graft survival as have already been performed, have been hampered by the differences in descriptive criteria employed in different studies, which utilise subjective assessments of disease severity (Rutherford & Becker, 1991). If future studies

involve the pre-operative determination of levels of thrombotic mediators this may provide objective assessment of disease severity, with a more reliable comparison between different series.

The development of a predictive risk index for the pre-operative assessment of patients being considered for infra-inguinal revascularisation, has the potential to improve on current assessment techniques, most of which are based on mechanical considerations alone (Parvin et al, 1985, Beard et al, 1989, Bell, 1991, Thompson et al, 1992), ignoring the potential contribution of the composition of the blood that will be required to flow through the graft, despite some evidence that altered levels of thrombotic mediators are associated with graft occlusion (Hamer et al, 1973, Wiseman et al, 1989). This thesis has considered the information routinely obtained pre-operatively in patients with PAOD, and on which the decision to operate is made. Multivariate analysis indicates that determination of pre-operative vWF levels provides important additional information, increasing the pre-operative ability to predict subsequent outcome in patients being considered for infra-inguinal revascularisation. The use of such a risk scoring system should therefore improve pre-operative patient selection, and reduce the number of early graft failures that leading to subsequent amputation, although it requires further validation before being adopted in routine clinical practice.

There was, in addition, evidence that prolonged post-operative elevations in vWF and perhaps also white cell count, were related to the development of vein graft stenosis in the patients studied, and this preliminary finding merits further investigation with a larger number of patients. More detailed assessment of white cell activation, involving determination of white cell activation markers such as neutrophil elastase, and interleukin-6, would perhaps provide further information in this situation.

Prolonged determination of levels of potential thrombotic mediators following successful infra-inguinal grafting indicated that some of the alterations associated with PAOD were only partly reversed by revascularisation, again implying that tissue ischaemia is not the sole cause of the abnormalities seen. In addition it is apparent that a significant increase in fibrin turnover persists following insertion of a synthetic graft. This indicates a dynamic deposition of fibrin within such a graft that is not seen with the use of autogenous vein, and in view of the potential of FDP's to enhance the development of atherosclerosis (Thompson et al, 1990, Lorenzet et al, 1992), together with their association with the progression of peripheral arterial disease, and with coronary events (Fowkes et

al, 1993) the use of such grafts, when autogenous vein is available, must be called into question.

Summary

This thesis has demonstrated that increased levels of the potential thrombotic mediators fibrinogen and vWF, together with increased fibrin turnover (as determined by levels of cross-linked fibrin degradation product levels) are related to the presence and severity of peripheral arterial occlusive disease. Revascularisation surgery fails to return the levels of these thrombotic mediators to normal population values, and elevated pre-operative von Willebrand Factor levels are predictive of post-operative graft occlusion and death. This association may be causal through an effect on intravascular thrombus formation and further studies are required to determine whether or not reducing the levels of potential thrombotic mediators will improve graft and patient survival. In addition a predictive risk index has been developed to assist in the identification of patients unlikely to benefit from infra-inguinal reconstruction, and in whom primary amputation may be more appropriate.

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B R I E F C O M M U N I C A T I O N

BLOOD RHEOLOGY, FIBRIN DEGRADATION PRODUCTS AND VON WILLEBRAND FACTOR IN ARTERIAL AND VENOUS BLOOD OF PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

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INTRODUCTION

A number of authors have related abnormalities of blood and plasma viscosity, fibrin degradation products (FDP), and markers of endothelial damage (von Willebrand Factor, vWF) in venous blood to peripheral and coronary arterial disease in man (1,2,3,4,5,6). Altered levels of these variables have also been accorded prognostic significance in some reports, and some may have a role in the pathogenesis of the lesions of atherosclerosis (7,8,9).

Although venous samples for estimation of these haemostatic and rheological variables are collected for such studies, it has never been shown that the results obtained from venous sampling accurately represent the haemostatic and rheological state encountered in arterial blood. In order to confirm that results obtained from venous sampling in patients with peripheral arterial disease, reflect the composition of the blood in their diseased arterial tree, we have compared blood viscosity, FDP levels, and vWF levels in peripheral venous blood samples, with those in arterial blood sampled from the vicinity of symptomatic arterial stenoses, in 14 patients undergoing percutaneous angioplasty.

MATERIALS AND METHODS

Fasting venous samples were obtained, without stasis, from the ante-cubital veins of 15 patients (mean age 63yrs, S.D.11yrs) with symptomatic peripheral arterial disease, using a 19-gauge needle. Diagnosis of peripheral vascular disease was based on an ankle-brachial pressure index (ABPI) of less than 0.85, together with a fall in the ABPI on walking, and a Seldinger arteriogram showing a stenosis of greater than 50% at a site appropriate to the symptoms. Arterial samples were obtained between 2 and 4 hours later at angioplasty, when samples of arterial blood were taken through a size 5 french angiography catheter, at the site

Key words: blood viscosity, fibrin degradation products, von Willebrand factor, peripheral arterial disease

of the arterial lesion (8 downstream of an iliac stenosis, 7 upstream from a superficial femoral lesion), prior to any interventional procedure being undertaken. Both arterial and venous samples were handled in an identical manner prior to being assayed.

Plasma and whole blood viscosity were measured at 37°C and high shear rates ($>300s^{-1}$) within 4 hours of sampling, in samples anticoagulated with K_2 EDTA (Monoject, Sherwood Medical, U.K.), using a Coulter capillary viscometer (Coulter Electronics, Luton, Beds. U.K.). Haematocrit was estimated by the Hawksley micro-centifuge method (Hawksley and Son Ltd., Lancing, W.Sussex, U.K.), and from these values the haematocrit-corrected blood viscosity was calculated (10).

Cross-linked FDP levels were measured using a commercially available ELISA test (Agen Ltd. Parsippany, New Jersey, U.S.A.), and von Willebrand factor estimation was carried out by ELISA (Dako, High Wycombe, U.K.). Both assays were performed on plasma obtained from 9ml blood anticoagulated with 1ml of 3.2% trisodium citrate, and centrifuged at 4 degrees centigrade for 15 mins. Plasma was stored at -70°C until assay, and both venous and arterial samples were assayed in the same batch to minimise inter-assay variation.

Control values for all variables were obtained from samples of venous blood obtained for this purpose from an age matched local population study.

All assays on arterial and venous samples were compared by Wilcoxon matched pairs test using the CCS:Statistica package (Statsoft, Tulsa, USA) on microcomputer.

RESULTS

In 2 of the 15 patients there was evidence of significant haemodilution in the arterial samples (as a result of flushing the angioplasty catheter with heparinised saline), with a greater than 25% fall in haematocrit between the 2 samples, and these patients were therefore excluded from further analysis. In the remaining 13 patients (Table 1) there were no significant differences in either plasma or whole blood viscosity between the venous samples, and the peri-lesional arterial sample, nor between arterial and venous levels of cross-linked F.D.P. However venous levels of von Willebrand Factor were significantly higher than in corresponding arterial samples ($p=<0.05$ Wilcoxon matched pairs test). There were no significant differences between the results obtained from superficial femoral or iliac samples.

TABLE 1

Comparison of arterial and venous samples in 13 patients with symptomatic peripheral arterial disease. Results are given as median values (interquartile range).

Variable	Mean venous level in population	Venous	Arterial
Plasma Viscosity (mPa.s)	1.32	1.30 (1.27-1.38)	1.28 (1.24-1.37)
Whole blood viscosity (mPa.s)	3.38	3.31 (3.11-3.56)	3.25 (3.06-3.40)
Haematocrit	44.2	45 (42-48)	44 (43-45)
Corrected blood viscosity (mPa.s)	3.41	3.31 (3.21-3.59)	3.29 (3.19-3.34)
F.D.P.s (ng/ml)	83	101 (82-126)	100 (78-149)
vWF (i.u./dl)	109	123 (113-147)	76 (56-101)

DISCUSSION

Altered blood viscosity in patients with peripheral arterial disease, has been demonstrated in a number of studies (1,2,3), and some authors have suggested that local alterations in rheological parameters may have a role in the distribution of the lesions of atherosclerosis (11). Pathological studies in patients with peripheral arterial disease have implicated fibrin deposition in the development of the arterial lesions (7,9), and in addition there is evidence implicating increased fibrin turnover in the disease process, elevated levels of cross-linked fibrin degradation products (F.D.P.) having been demonstrated in patients with symptomatic peripheral arterial disease (6,12).

These findings have however all been made in venous blood samples, taken from the antecubital vein, and in view of the focal nature of the lesions of atherosclerosis, the situation in arterial blood may be different. Our results however indicate that venous sampling represents an accurate reflection of the composition of arterial blood in patients with symptomatic peripheral arterial disease, with the exception of von Willebrand Factor antigen levels, which appear to be consistently slightly lower in arterial blood. The reason for this difference is not known, but may reflect decreased production from a damaged arterial endothelium at sites of stenosis.

The lack of any significant difference in blood and plasma viscosity between arterial and venous samples confirms that the elevated viscosity encountered in venous blood in patients with advanced peripheral arterial disease is present in arterial blood also, where, in the presence of arterial stenoses, it is likely to make a significant contribution to altered flow dynamics.

The similarity between arterial and venous levels of cross-linked F.D.P. levels indicates that any contribution that altered fibrin turnover makes to peripheral arterial disease, and in particular to the progression of atherosclerotic plaques (7,9), is not made by local alterations in fibrin turnover at the sites of development of symptomatic stenoses. The elevated levels of F.D.P. levels that have been documented in peripheral arterial disease (6,12,14), appear to reflect a systemic alteration in fibrin turnover.

In summary our results refute any suggestion of local alterations in haemostatic and rheological parameters at the sites of symptomatic lesions in patients with peripheral arterial disease, and confirm that venous blood sampling provides an accurate reflection of the composition of arterial blood in such patients, although it consistently overestimates the concentration of vWF antigen present in arterial blood samples taken from the vicinity of a symptomatic stenosis. Further studies are required to study the nature of this arterio-venous difference.

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